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Copper Sensitivity, Host Resistance, and Bacteriophage Biocontrol of *Xanthomonas euvesicatoria* pv. *euvesicatoria*, the Causal Agent of Bacterial Spot in Pepper and Tomato Plants in the Riyadh Region, Saudi Arabia

Talha Mansoor, Yasser E. Ibrahim, Ali A. Al-Masrahi, Abdullah F. Alhashel, and Mohammed A. Al-Saleh

Department of Plant protection, College of Food and Agricultural sciences, King Saud University, P.O. Box 2460, Riyadh 11451, Saudi Arabia

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Abstract. **Bacterial spot disease, caused by *Xanthomonas euvesicatoria* pv. *euvesicatoria* significantly threatens tomato and pepper production globally, including in Saudi Arabia. This study evaluated the sensitivity of 85 *X. e.* pv. *euvesicatoria* strains from Riyadh to copper sulfate, zinc sulfate, and streptomycin in laboratory and greenhouse experiments. All *X. e.* pv. *euvesicatoria* strains demonstrated a positive reaction (growth or resistance) at lower concentrations of copper sulfate (50 and 100 μg/mL). Still, some strains exhibited certain degrees of resistance patterns to higher concentrations of copper sulfate (200 and 400 μg/mL). Saudi Arabian strains demonstrated some variability in their response to zinc sulfate. Sensitivity to streptomycin was consistent across all strains. The population dynamics of *X. e.* pv. *euvesicatoria* were further assessed in six tomato and pepper cultivars. Pasheeen Plus F1 and Pepper Galileo were moderately susceptible among peppers, whereas Basha F1 demonstrated greater tolerance. Tomato Jawaher exhibited high resistance in tomatoes, with bacterial populations restricted to 8×10^{10} CFU/cm^2. In addition, bacteriophages were investigated as eco-friendly biocontrol agents against *X. e.* pv. *euvesicatoria*. Phage-based treatments significantly (86.4%) reduced bacterial populations and average lesion number in greenhouse trials, with the greatest effectiveness observed when combined with copper hydroxide. Phages in a concentration of 1×10^{10} plaque-forming units/mL demonstrated high specificity for infecting and controlling *X. e.* pv. *euvesicatoria*. These results indicate that the sustainable development of bacteriophage therapy as an alternative to traditional pesticides may prevent damage from bacterial spot on plant leaves while mitigating issues such as chemical resistance or environmental hazards.**

Bacterial spot (BS), which can be caused by four *Xanthomonas* species (*X. vesicatoria*, *X. euvesicatoria* pv. *euvesicatoria*, *X. e.* pv. *perforans*, and *X. cynarae.* pv. *gardneri*), is an serious disease, threatening the tomato (*Solanum lycopersicum*) and pepper (*Capsicum* spp.) industries globally (Potnis et al. 2015). In Saudi Arabia, *X. e.* pv. *euvesicatoria* and *X. vesicatoria* are the predominant pathogens, with the latter identified as the primary cause of BS (Ibrahim and Al-Saleh 2011, 2012). BS bacteria pose significant challenges under field and greenhouse conditions due to their adaptability and dispersal ability (Ibrahim and Al-Saleh 2012). Under conducive environmental conditions (high rainfall, higher temperatures, and extended leaf wetness), the disease causes severe yield losses. For example, surveys identified 10% to 95% reductions in yield in Turkey and North Macedonia (Aysan and Sahin 2003; Mitrev and Kovačević 2006). Traditionally, BS management strategies have relied on chemical control and cultural practices (Potnis et al. 2015). Since the 1950s, antibiotics and some copper-based substances have been used to control BS (Thayer and Stall 1962). Nevertheless, the frequent use of these compounds has resulted in the frequent emergence of copper-resistant *Xanthomonas* strains worldwide, including (Abbasi et al. 2015; Araújo et al. 2012; Klein-Gordon et al. 2021; Klein-Gordon et al. 2022; Marco and Stall 1983; Mirik et al. 2007). Combining mancozeb and copper-based bactericides has been recommended to enhance BS management. However, these combinations did not improve BS management resulting from copper-resistant strains, particularly under environmental conditions conducive to disease development (Jones and Jones 1985). The accumulation of copper in soil and groundwater may adversely affect the ecosystem and plant health, potentially resulting in unfavorable subsequent crops (Yoon et al. 2006). Developing host plant resistance is a sustainable, long-term solution for managing. Though none of the tomato cultivars have proven completely resistant to the various *Xanthomonas* species responsible for BS, there has been evidence that partial resistance involving multiple genes provides broader-spectrum protection and reduces the risk of resistance breakdown (Scott et al. 2006; Sharma and Bhattarai 2019; Stall et al. 2009). In the case of tomato and pepper, screening for hypersensitive resistance genotypes can additionally contribute to their disease resistance (Astua-Monge et al. 2000; Jones and Scott 1986). Acibenzolar-S-methyl (ASM) has emerged as a promising compound for controlling BS (Pontes et al. 2016). It induces systemic acquired resistance (SAR) in plants. Bacteriophages present a promising biocontrol approach. Bacteriophages are viruses that infect bacteria but do not damage other beneficial microbes, which is why they are eco-friendly substitutes for chemical pesticides (Holtappels et al. 2021; Jones et al. 2012). Bacteriophage cocktails have effectively controlled several bacterial pathogens, including *X. citri* pv. *citri*, *Dickeya solani*, *Pectobacterium carotovorum*, and *Ralstonia solanacearum* (Bertolini et al. 2023; Ibrahim et al. 2017; Petrzik et al. 2023; Zaczek-Moczydłowska et al. 2020). For example, jumbo phages like XaC1 and XbC2 have shown specificity for *X. vesicatoria* and thermal stability in the context of agricultural conditions, making them promising candidates for field use (Villicaña et al. 2024). Additionally, the integration of bacteriophages in copper-based control schemes could improve their effectiveness while reducing the likelihood of resistance development (Lang et al. 2007; Šević et al. 2019). Phage-based treatments combined with copper hydroxide or SAR inducers have shown a synergistic effect, resulting in reduced disease severity in field and greenhouse trials (Jones et al. 2004; Lang et al. 2007; Šević et al. 2019). This study aimed to investigate the sensitivity of 85 Saudi *X. e.* pv. *euvesicatoria* strains isolated from tomato and pepper plants from the Riyadh region of Saudi Arabia to copper sulfate, zinc sulfate, and streptomycin. The

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Y.I. is the corresponding author. E-mail: yasereid@ksu.edu.sa.

susceptibility of six local cultivars of tomato and pepper was also examined to assess genotypes possibly resistant to the pathogen eventually be included in sustainable production systems. Commercial phage-based solutions for decreasing BS are offered in the United States, yet their effectiveness against local pathogen populations is unknown (Balogh et al. 2008). Moreover, the effectiveness and longevity of these products rely on an ongoing phage prospecting process that results in distinct pathogen assembly and predominant species in given fields. In this study, we isolated and characterized bacteriophages that are used to manage BS on pepper in Saudi Arabia. We investigated the prevalence, host range, and in vivo properties of four X. *e.* pv. *euvesicatoria*–specific bacteriophages as biological control agents on pepper seedlings cultivated under greenhouse conditions. Obtaining the aforementioned insights (pathogen diversity dynamics, resistance evolution, and sustainable crop protection practices against BS in tomato and pepper crops in Saudi Arabia) is intended to advance integrated management strategies for BS.

Materials and Methods

Screening for copper, zinc, and streptomycin sensitivity. Eighty-five strains of *X. e.* pv. *euvesicatoria* was used in this study. These strains were isolated from the leaves and fruits of pepper and tomato plants exhibiting symptoms of BS in various regions of Riyadh, Saudi Arabia, and identified using biochemical and molecular analysis using species-specific primers (one new primer set, Euv8, developed by us) and multilocus sequence analysis using the sequences of *fusA* and *gltA* housekeeping genes. From the 85 Saudi *X. e.* pv. *euvesicatoria* isolates, a subset of nine isolates were selected based on a pathogenicity test (Ibrahim et al. 2025, unpublished data) to represent both host plants (tomato and pepper) and regions where infestations were recorded, and their sequences were deposited in GenBank (Table 1). The sensitivity of the Saudi strains to copper sulfate (Sigma-Aldrich, St. Louis, MO, USA), zinc sulfate (Ecibra, São Paulo, Brazil), and streptomycin sulfate (Calbiochem) (Sigma-Aldrich) was tested at concentrations of 0, 50, 100, 200, and 400 µg/mL. For copper and zinc assays, a sterilized sucrose peptone agar (SPA) medium (Hayward 1960) was prepared with the desired concentrations of each compound, while PSA without additives served as the control. Streptomycin assays were conducted similarly, using filter-sterilized (0.45 µm; Millipore, Burlington, MA, USA) streptomycin stock solutions. Individual pure cultures of the strains were grown on nutrient agar (Scharlau, Barcelona, Spain) supplemented with 0.5% sucrose agar (NSA) at 28 °C for 48 h. Bacterial suspensions were prepared in sterile distilled water (SDW) and adjusted to a turbidity corresponding to 10^8 CFU/mL (A_{600} = 0.3). Concentrations of *X. e.* pv. *euvesicatoria* viable cells were confirmed by dilution plating on nutrient agar plates. Plates were incubated at 28 °C for 36 to 72 h, and bacterial growth was recorded.

Cultivar resistance evaluation. Three sweet pepper cultivars (Pasheeen Plus F1, China; Galileo, United States; Basha F1, United States) and three tomato cultivars (Dareen F1, Kenya; Tone Guitar F1, United States; and Jawaher, United States) were evaluated for resistance. Seeds were grown in 10 cm (51 mL) pots containing sand: peatmoss substrate (2:1 v/v), sterilized by autoclaving. Pepper and tomato plants were grown in a greenhouse at 23 to 28 °C, fertilized, and watered as needed. Bacterial strain *X. e.* pv. *euvesicatoria* (GGH02) was cultured on NSA for 72 h (Robinson et al. 2006). Bacterial suspensions were adjusted to 1×10^5 CFU/mL in phosphate-buffered saline (PBS). Leaves of 5-week-old plants were infiltrated with ~0.03 mL of the suspension using a needleless syringe, and the inoculated plants were maintained under greenhouse conditions described earlier. Samples for bacterial population assessments were taken at 0, 3, 5, 8, 10, and 14 d post-inoculation (dpi). Three replications were implemented for each cultivar, and each replicate consisted of 10 plants. The experiment was performed in duplicate.

Bacteriophage isolation. Pepper and tomato leaf tissues with characteristic symptoms of BS were disinfected with 70% ethanol for 30 s and then washed three times in SDW. The pepper and tomato leaf infected tissue samples, consisting of 10 g, were placed in 250-mL flasks containing 50 mL SDW and shaken for 30 min at 250 rpm/min. Two milliliter aliquots were collected and centrifuged for 15 min at 10,000 g_n. The supernatants were passed through a 0.45-µm membrane filter, and the resulting filtrates were stored in microfuge tubes in complete darkness at 4 °C. Isolated bacteriophages were mixed with the copper-sensitive bacterial strain GGH02, and then the mixture was spread on plates containing soft nutrient agar medium (10 g/L). After a 24-h incubation period at 28 °C, if lysis was observed, the phage was purified using two separate single-plaque isolation procedures (Mohammadi and Ely 2024). To infect *X. e.* pv. *euvesicatoria,* bacteria were cultivated in semisolid nutrient agar-yeast extract medium (NYA) [0.8% NB, 0.6% Bacto agar (Difco), and 0.2% Yeast Extract (Difco)], four morphologically different plaques were selected and mixed. The bacteriophage mixture that was propagated was kept at 4 °C in total darkness in SM (sodium chloride/magnesium sulfate) buffer (0.05 M Tris-HCl, pH 7.5, 0.1 M NaCl, 10 mM $MgSO_4$, and 1% gelatin). The final phage suspension was prepared using either SM buffer or SDW. Serial dilutions were used to determine phage concentrations after 24 to 48 h. The phage concentration was calculated from the plaque number, and specific dilution was expressed as plaque-forming units (PFU)/milliliter (Rizvi and Mora 1963).

Host range test. The host range of purified bacteriophages was evaluated against the nine *X. e.* pv. *euvesicatoria* strains (Table 1) and local strains of *Clavibacter michiganensis* subsp. *michiganensis*, *Erwinia amylovora*, and *Xanthomonas citri* pv. *citri*. Bacterial lawns were prepared on King's B agar (King et al. 1954), and 10-µL phage suspensions were spotted onto the bacterial lawn. The presence of a lysis zone at the site where the phage sample was deposited for each bacterial isolate was interpreted to indicate sensitivity, and the absence of a lysis zone to indicate resistance to phage infection. The experimental design was fully randomized in a factorial experiment (bacteriophages × isolates of bacteria), and each treatment had three replications.

Greenhouse evaluation of phage-based treatments. Greenhouse trials were conducted in a randomized complete block design to evaluate bacteriophage efficacy. Twenty-day-old pepper seedlings of cv. Early California Wonderful, with two true leaves, were transplanted into 2-L pots filled with autoclaved soil and kept in a greenhouse with humidity and temperature control. One week after transplanting, the treatments were applied when the plants had four true leaves. The treatments were as follows: 1) untreated control (water only), 2) copper hydroxide (5 g/L) applied every week; 3) a mixture of four bacteriophages diluted to a final concentration of 1×10^{10} PFU/mL applied every 5 d; and 4) a mixture of bacteriophages + copper hydroxide at a recommended concentration (5 g/L). Treatments were applied by spraying to runoff using a compressed CO_2sprayer. One week after initiating treatments, seedlings were inoculated with *X. e.* pv. *euvesicatoria* strain GGH02 at 10^6 CFU/mL. Plants were sealed within plastic bags and placed in a greenhouse at 28 °C and a 12-h photoperiod for 48 h. The plastic bags were removed after 48 h, and disease incidence was assessed at 7, 14, and 21 dpi by counting BS lesions on five

Table 1. *Xanthomonas euvesicatoria* pv. *euvesicatoria* strains evaluated in this study.

Location/host	Strain	Accession No. *fusA*	Accession No. *gltA*	Accession No. Euv8
GOF/tomato	SA-01	PP767866	PP767875	PP767884
GGH/tomato	SA-02	PP76867	PP767876	PP767885
GGH/pepper	SA-03	PP767868	PP767877	PP767886
KOF/tomao	SA-04	PP767869	PP767878	PP767887
KGH/tomato	SA-05	PP767870	PP767879	PP767888
KGH/pepper	SA-06	PP767871	PP767880	PP767889
ZOF/tomato	SA-07	PP767872	PP767881	PP767890
ZOF/pepper	SA-08	PP767873	PP767882	PP767891
ZGH/pepper	SA-09	PP767874	PP767883	PP767892

G = Ghat; K = Kharj; Z = Zulfi; T = tomato; P = Pepper; OF = open field; GH = greenhouse.

Table 2. Sensitivity of 85 Saudi Arabian strains of *Xanthomonas euvesicatoria* pv. *euvesicatoria* against different concentrations of copper sulfate, zinc sulfate, and streptomycin.

Product	Concn ($\mu g \cdot mL^{-1}$)	Strain/location Al Ghat (36)[i]	Al-Kharj (33)[i]	Az Zulfi (16)[i]
Copper sulfate	50	100[ii]	100	100
	100	100	100	100
	200	5.6	0	12.5
	400	5.6	0	12.5
Zinc sulfate	50	100	100	100
	100	100	100	100
	200	2.7	0	0
	400	2.7	0	5.4
Streptomycin	50	0	0	0
	100	0	0	0
	200	0	0	0
	400	0	0	0

[i] Number of strains analyzed.
[ii] Tolerant strains %.

leaves per plant. Lesions from each treatment were sampled, and the isolated bacteria were confirmed to be *X. e.* pv. *euvesicatoria* using our Euv8-specific primer. Disease incidence was calculated as the percentage of diseased leaves with BS symptoms per plant. The area under the disease progress curve (AUDPC) was calculated following Shaner and Finney (1976). Five replications were implemented for each treatment, consisting of 10 plants. The experiment was performed in duplicate.

Statistical analysis. Data from greenhouse trials were pooled when Bartlett's test indicated no significant differences ($P > 0.05$). The number of lesions and AUDPC values were analyzed using one-way analysis of variance (ANOVA; SAS version 9.1), and treatment means were compared using Fisher's least significant difference test at $P < 0.05$.

Phage population analysis. Leaf samples were collected from the pepper leaves treated with a mixture of four bacteriophages and inoculated with *X. e.* pv. *euvesicatoria* in the greenhouse evaluation described above. The leaves were put inside a zipper-seal plastic freezer bag and brought to the laboratory. In the laboratory, the weight of each bag was obtained one by one, and 100 mL of deionized water was added to the individual bags. After shaking the bags for 15 min, 1 mL of the rinsate was transferred to 1.5 mL microcentrifuge tubes. Then, 100 µL of chloroform was added to each microcentrifuge tube, which was placed on a rotating shaker for 30 min. After incubation, the chloroform was pelleted via a pulse spin in a microcentrifuge, and 700 µL of the supernatant was transferred into a sterile microcentrifuge tube. A tube was then centrifuged for 15 min at 30,678 g_n to remove cellular debris. The phage titer was quantified after a dilution series of the supernatant. For enumeration, 24-h-old cultures were removed from agar plates and resuspended in SDW. One hundred microliters of the phage solution was added to a sterile 90-mm-diameter petri dish and supplemented with 100 µL of the suspension of the host bacterium. Finally, 16 mL of a nutritional broth NYA medium [0.8% (wt/vol) nutritional broth; 0.6% (wt/vol) Bacto Agar, 0.2% (wt/vol) yeast extract], kept at 48 to 50 °C, was added. The growth medium was shaken thoroughly in the petri dish to mix the bacterial cells and phage virions. The plate was incubated for 2 to 3 d at 28 °C until the bacterial lawn developed and the plaques were visible. Enumeration of the plaques was performed at suitable dilutions. The phage titer was calculated as the number of PFU per gram of leaf tissue according to the following equation: y = plaque number × 1000 (100 µL of the 100 mL original volume plated)/dilution ratio/[sample bag weight – empty bag weight (g)]. Data obtained from each sample were analyzed for statistical purposes after log transformation of the data [z = log10(y + 1)], followed by ANOVA and Fisher's multiple range test.

Results

Screening for copper, zinc, and streptomycin sensitivity. The tolerance of Saudi Arabian *Xanthomonas* strains isolated from three tomato and pepper producing regions—Al Ghat (36 strains), Al-Kharj (33 strains), and Az Zulfi (16 strains)—was evaluated against copper sulfate, zinc sulfate, and streptomycin at increasing concentrations (50, 100, 200, and 400 µg/mL). At lower concentrations (50 and 100 µg/mL), all Saudi strains (100%) from the three locations exhibited complete tolerance to copper sulfate and zinc sulfate (Table 2). However, tolerance dramatically declined at higher concentrations. At 200 µg/mL of copper sulfate, only 5.6% of strains from

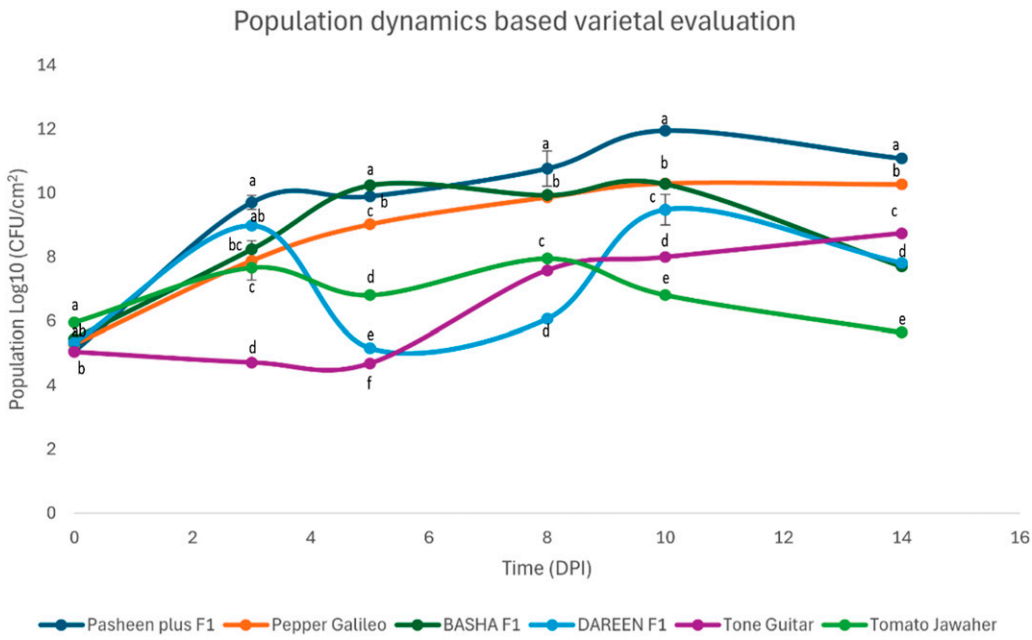

Fig. 1. Bacterial populations in pepper and tomato cultivars leaves over 2 weeks after infiltration with *Xanthomonas euvesicatoria* pv. *euvesicatoria* 10^5 CFU/mL. Means and standard error bars are shown for six cultivars for each cultivar at each sampling interval for three replicates of each host–bacterium combination at each sampling time.

Table 3. Host range of phages against Saudi Arabian *Xanthomonas euvesicatoria* pv. *euvesicatoria* strains and other bacterial species.

	Saudi Arabian bacterial strains (*X. e.* pv. *euvesicatoria*)									Others		
Phage	Sa-01	Sa-02	Sa-03	Sa-04	Sa-05	Sa-06	Sa-07	Sa-08	Sa-09	*Erwinia amylovora*	*Xanthomonas citri* pv. *citri*	*Clavibacter michiganensis* sub sp. *michiganensis*
Phage 1	+	+	+	+	+	+	+	+	+	−	−	−
Phage 2	+	+	+	+	+	+	+	+	+	−	−	−
Phage 3	+	+	+	+	+	+	+	+	+	−	−	−
Phage 4	+	+	+	+	+	+	+	+	+	−	−	−

− no plaque; + clear plaque.

Al Ghat and 12.5% from Az Zulfi remained tolerant, whereas no tolerant strains were detected from Al-Kharj. The same trend was observed at 400 µg/mL. For zinc sulfate at 200 µg/mL, tolerance dropped to 2.7% in Al Ghat and was absent in Al-Kharj and Az Zulfi. At 400 µg/mL, only Al Ghat and Az Zulfi showed minimal tolerance (2.7% and 5.4%, respectively). In contrast, no tolerance to streptomycin was noted at any concentration tested in any location (Table 2). All strains were susceptible, indicating uniform sensitivity of the bacterial populations to this antibiotic.

Cultivar resistance evaluation. The population dynamics of *X. e.* pv. *euvesicatoria* differed significantly among the six cultivars tested for pepper and tomato. The highest susceptibility was recorded for pepper cvs Pasheeen Plus F1 and Galileo in the fourth week post-inoculation, as bacterial populations reached the maximum at 12–12.5× 10^{10} CFU/cm^2 by 8 DPI and remained stable afterward (Fig. 1). However, pepper cv. Basha F1 showed moderate resistance with around 10^{10} CFU/cm bacterial load at peak, which declined after 12 DPI. Tomato cvs. Dareen and Tone Guitar showed moderate susceptibility among tomato cultivars, with speaks at 11 and 11.5 × 10^{10} CFU/cm^2, followed by a gradual decline. In contrast, tomato cv. Jawaher Tomato showed the highest resistance with low and consistently low populations peaking at only 8^{10} CFU/cm^2. The results demonstrated significant differences in bacterial multiplication between the two cultivars, identifying Tomato Jawaher and Basha F1 as the most resistant in their respective species.

Host range of phages when tested against nine Saudi Arabian X. e. *pv.* euvesicatoria *strains and a strain each of three other bacterial species* The host range of four phages tested against nine *X. e.* pv. *euvesicatoria* strains isolated from Saudi Arabia and three other bacterial species are shown in Table 3. All four phages formed clear plaques (+) on the *X. e.* pv. *euvesicatoria* strains tested (SA-01 to SA-09), demonstrating their ability to lyse these bacterial strains. In contrast, all phages exhibited no activity (−) against a strain of *Erwinia amylovora*, and *Xanthomonas citri* pv. *citri*, or *Clavibacter michiganensis* subsp. *michiganensis*.

Effect of bacteriophage on BS disease development under greenhouse conditions. Table 4 shows the impact of treatments on the development of BS, taking the average lesion number and the AUDPC into account. The strongest effect was obtained (with the least average number of lesions of 3.1 and AUDPC value of 29.8) for the combined treatment of phage and copper hydroxide. The application of the mixture of phages alone or copper hydroxide alone had moderate protection against the infection, with an average number of lesions of (6.6 and 13.3) and an AUDPC of (34.6 and 45.0), respectively.

Phage population analysis. Phage populations on pepper leaf foliage progressively dropped over the 7 d after application (Fig. 2). On day 1, titers reached ∼6.5 log units and remained statistically unchanged on day 2 ($P >$ 0.05). A significant reduction was observed on days 3 and 4, with phage levels dropping to ∼5.5 units ($P < 0.05$). By day 5, titers decreased to ∼4.8 units, followed by a marked decline on days 6 and 7 to ∼4.2 and 3.6, respectively. Means followed by different letters are significantly different, indicating statistically significant changes in population over time. These findings demonstrate that phage viability on pepper leaf foliage declines significantly within 1-week post-application.

Discussion

This study assessed the sensitivity of 85 Saudi Arabian *X. e.* pv. *euvesicatoria* strains from tomato and pepper plants to copper sulfate, zinc sulfate, and streptomycin while evaluating the potential of bacteriophage-based biocontrol strategies. The findings reveal varying resistance levels to these compounds, with copper sulfate exhibiting the most pronounced inhibitory effects at higher concentrations (200–400 µg/mL). The observed resistance to copper and zinc sulfates aligns with earlier studies that reported high percentages of *Xanthomonas* spp. resistance to 200 µg/mL copper sulfate and 100 µg/mL zinc sulfate in tomato and pepper plants (Ritchie and Dittapongpitch 1991; Ward and O'Garro 1992). The high resistance to copper sulfate is likely attributed to the prolonged and intensive use of copper-based fungicides, indicating prevalent practices in Saudi Arabia that have led to the selection of resistant strains (Aguiar et al. 2000; Ward and O'Garro 1992). Similar resistance trends have been documented in *Xanthomonas* populations from Brazil, the United States, Canada, and Tanzania (Abbasi et al. 2015; Shenge et al. 2014; Thayer and Stall 1962). This highlights the global challenge posed by copper-resistant strains in agricultural disease management. Resistance to streptomycin was widespread, with all strains showing

Table 4. Effect of phage on bacterial spot disease development on pepper in the greenhouse.

Treatments	Avg lesion number[i]	AUDPC
Phage mixtures	6.6 c	34.6 c
Phage + copper hydroxide	3.1 c	29.8 c
Copper hydroxide	13.3 b	45.0 b
Untreated control	22.8 a	61.3 a

[i] Average lesion number per leaf 21 d after inoculation.

Mean separation within columns by Fisher's least significant difference, $P = 0.05$ level. Means followed by different letters within a column are significantly different. AUDPC = area under the disease progress curve.

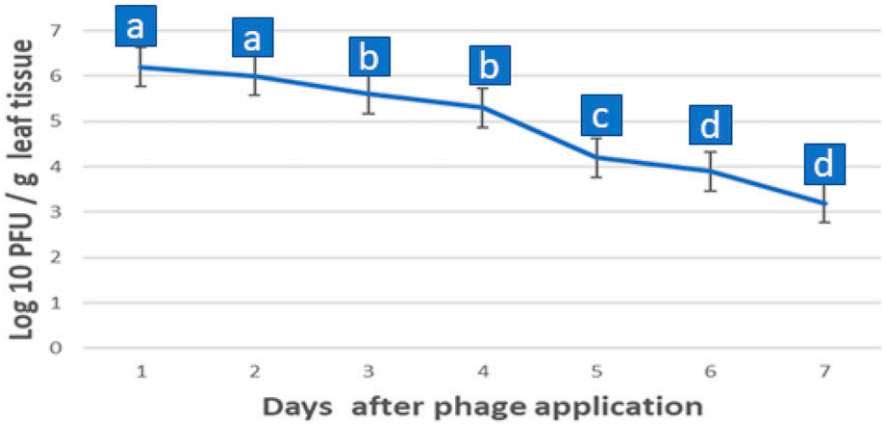

Fig. 2. Changes in bacteriophage populations on pepper foliage during 7 d in greenhouse conditions. Error bars indicate the standard error.

minimal inhibition even at higher concentrations, consistent with previous reports of streptomycin-resistant *Xanthomonas* strains globally (Bouzar et al. 1999; Vallad et al. 2013).

The bacterial multiplication studies across tomato and pepper cultivars revealed significant variability in host susceptibility. Tomato Jawaher and Basha F1 demonstrated strong and moderate resistance, respectively, while Pasheeen Plus F1 and Pepper Galileo were the most susceptible. These findings are consistent with previous studies linking host resistance to expressing disease-resistance genes, such as *Bs2*, and partial resistance mechanisms like reduced bacterial colonization (Schornack et al. 2004; Timilsina et al. 2020). These significant results suggest that breeding programs that emphasize improving genetic resistance of *X. e.* pv. *euvesicatoria* in both crops should be further enhanced, where cultivars such as tomato Jawaher and Basha F1 could be valuable resources for integrated disease management strategies with sustainability.

Several reports have documented phages infecting members of the *Xanthomonas* genus (Ibrahim et al. 2017; Nakayinga et al. 2021; Villicaña et al. 2024); however, some of these studies showed that the phages have a limited host range, infecting only strains within the same species. This presents a challenge for managing BS of tomato using phage therapy because the disease can be caused by up to four distinct *Xanthomonas* species (Osdaghi et al. 2021).

In the current investigation, all four phages isolated exhibited no activity (−) against *E. amylovora*, *X. c.* pv. *citri*, and *C. m.* subsp. *michiganensis*. These results indicate that the phages are possibly highly specific for *X. e.* pv. *euvesicatoria* strains and can potentially be employed as biocontrol agents for managing BS disease in Saudi Arabia.

The selectivity of bacteriophages, which would target only *X. e.* pv. *euvesicatoria* and no other (related) species adds yet another means as a substitute for broad-spectrum chemical bactericides. However, the narrow host range of bacteriophages means that phage cocktails must be developed against strains of each of the four species causing BS.

Environmental factors, such as ultraviolet radiation and temperature, can impact phage persistence on plant surfaces, requiring seasonal re-isolation to maintain efficacy (Holtappels et al. 2021; Jones et al. 2012). The findings from this study indicate that bacteriophage therapy, particularly in combination with copper hydroxide, holds great promise for managing BS caused by *X. e.* pv. *euvesicatoria*. This combination of phage application with copper hydroxide showed synergistic effects, resulting in significant reductions in the AUDPC (up to 60.3%). These findings align with previous reports on the effectiveness of bacteriophage–copper combinations in reducing BS severity on tomato (Lang et al. 2007; Obradovic et al. 2004; Šević et al. 2019). Additionally, although phages offer advantages in reducing the development of resistance, integrating them with host-resistant cultivars, such as Tomato Jawaher and Basha F1, could provide a more robust and sustainable disease management strategy. Furthermore, this study documents the first record of Saudi Arabian *X. e.* pv. *euvesicatoria* strain's sensitivity and resistance to commonly used chemical compounds, contributing valuable insights into regional pathogen control strategies. Future research should focus on field trials across regions with diverse growing conditions to validate greenhouse findings and explore the scalability of phage-based biocontrol strategies. Moreover, understanding the molecular mechanisms of resistance and host-pathogen interactions will be crucial for optimizing integrated pest management programs and ensuring long-term agricultural sustainability of control measures against BS.

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