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Trishna Rawal
Department of Plant Health Management, Navsari Agricultural University, Navsari, Gujarat, India

Omprakash Basumatary
Department of Plant Health Management, Navsari Agricultural University, Navsari, Gujarat, India

Uday Rana
Department of Agricultural Microbiology, Sardarkrushinagar Dantiwada Agricultural University, Dantiwada, Gujarat, India

Corresponding Author:
Trishna Rawal
Department of Plant Health Management, Navsari Agricultural University, Navsari, Gujarat, India

Characterization of xanthomonas strains causing bacterial leaf spot in capsicum

Trishna Rawal, Omprakash Basumatary and Uday Rana

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Abstract
Bacterial leaf spot (BLS) caused by *Xanthomonas* spp. destroys 20–60% of capsicum yield annually in western India, yet the pathogen's strain-level diversity in Gujarat remains poorly documented. This research characterised seven *Xanthomonas* isolates collected from symptomatic capsicum fields across Navsari and Dantiwada districts during the 2022 kharif season. Morphological, biochemical (LOPAT), molecular (16S rDNA sequencing), and pathogenicity tests were performed at Navsari Agricultural University. All seven isolates were confirmed as *Xanthomonas euvesicatoria*. Disease severity on five capsicum cultivars ranged from 1.1 to 4.9 on a 1–5 scale. Strains XCV-02 and XCV-05 were the most aggressive, producing disease scores above 4.0 on susceptible cultivars California Wonder and Arka Mohini. Antibiotic sensitivity tests showed that streptomycin sulphate at 500 ppm gave the widest inhibition zones. The findings provide a strain-level baseline for designing targeted resistance breeding and chemical management strategies against BLS in Gujarat.

Keywords: *Xanthomonas euvesicatoria*, bacterial leaf spot, capsicum, strain characterization, pathogenicity, LOPAT test, 16S rDNA, antibiotic sensitivity, disease severity

Introduction
Bacterial leaf spot of capsicum (*Capsicum annuum* L.) has become the most damaging foliar disease in the warm, humid growing zones of western India, yet farmers and extension workers often treat it as a single pathogen problem with a blanket copper spray [1, 2]. The reality is more complicated. At least four *Xanthomonas* species *X. euvesicatoria*, *X. vesicatoria*, *X. perforans*, and *X. gardneri* can cause nearly identical leaf spot symptoms, and each species may harbour strains that differ in virulence, host range, and fungicide sensitivity [3, 4].

Without strain-level identification, resistance breeding targets the wrong pathotype and chemical recommendations may be ineffective [5]. Molecular tools, particularly 16S rDNA sequencing, have made it possible to assign isolates to species with high confidence, while standardised pathogenicity assays on differential cultivars reveal virulence variation within a species [6, 7].

Gujarat is one of India's largest capsicum-producing states, but no published work has characterised the *Xanthomonas* population on capsicum at the strain level in this region [8]. This research aimed to: (a) isolate and identify *Xanthomonas* strains from BLS-affected capsicum fields in Gujarat, (b) assess virulence on five commercial cultivars, and (c) test antibiotic sensitivity to guide chemical management recommendations.

Reagents and Chemicals
Nutrient glucose agar (NGA) was prepared from bacteriological peptone (HiMedia, Mumbai), yeast extract, and dextrose. LOPAT reagents levans sucrose agar, oxidase discs, pectate gel medium, arginine dihydrolase broth, and tobacco leaf infiltration supplies were procured from HiMedia. For molecular work, a genomic DNA extraction kit (Qiagen DNeasy Plant Mini Kit), universal 16S primers 27F and 1492R (Sigma-Aldrich, Bengaluru), Taq polymerase, dNTPs, and agarose (SRL Chemicals) were used. Antibiotic discs of streptomycin sulphate (100, 300, 500 ppm), copper oxychloride (0.2% and 0.3%), and kasugamycin (200 ppm) were prepared in-house following standard disc-diffusion protocol [6]. All chemicals were analytical grade and stored per manufacturer guidelines.

Material and Methods

Material: Symptomatic leaves showing water-soaked lesions with chlorotic halos were collected from seven capsicum fields across Navsari (20.95° N, 72.93° E) and Dantiwada (24.32° N, 72.19° E) during the 2022 kharif season (July–October). Each isolate was designated XCV-01 through XCV-07. Five capsicum cultivars California Wonder, Pusa Jwala, Arka Mohini, Kashi Anmol, and Punjab Lal served as differential hosts. The research was conducted at the Plant Pathology Laboratory, Navsari Agricultural University.

Methods: Leaf pieces (3 × 3 mm) from lesion margins were surface-sterilised with 1% NaOCl, plated on NGA, and incubated at 28 °C for 48 h. Yellow, mucoid colonies were purified by streaking and subjected to LOPAT tests [3]. DNA was extracted and 16S rDNA amplified by PCR (27F/1492R), sequenced (Sanger method), and compared against NCBI GenBank using BLASTn. Pathogenicity was confirmed by infiltrating bacterial suspension (10^8 CFU mL^{-1}) into leaves of 45-day-old potted plants of all five cultivars; disease severity was scored at 14 days post-inoculation on a 1–5 scale [7]. Antibiotic sensitivity was measured by the disc-diffusion method, recording inhibition zone diameter after 48 h at 28 °C. Each test included three replications; data were analysed by ANOVA and Tukey's HSD at $p \leq 0.05$.

Results

Table 1: Morphological and molecular identification of seven Xanthomonas isolates from capsicum in Gujarat

Isolate	Colony colour	LOPAT profile	16S rDNA match	Identity (%)	GenBank acc.	Pathogenicity
XCV-01	Yellow	−+−−−	*X. euvesicatoria*	99.3	OQ847231	+
XCV-02	Yellow	−+−−−	*X. euvesicatoria*	99.7	OQ847232	+
XCV-03	Yellow	−+−−−	*X. euvesicatoria*	98.9	OQ847233	+
XCV-04	Yellow	−+−−−	*X. euvesicatoria*	99.1	OQ847234	+
XCV-05	Yellow	−+−−−	*X. euvesicatoria*	99.6	OQ847235	+
XCV-06	Pale yel.	−+−−−	*X. euvesicatoria*	98.7	OQ847236	+
XCV-07	Yellow	−+−−−	*X. euvesicatoria*	99.2	OQ847237	+

LOPAT: Levan, Oxidase, Pectolysis, Arginine, Tobacco HR. + = positive pathogenicity on at least one cultivar

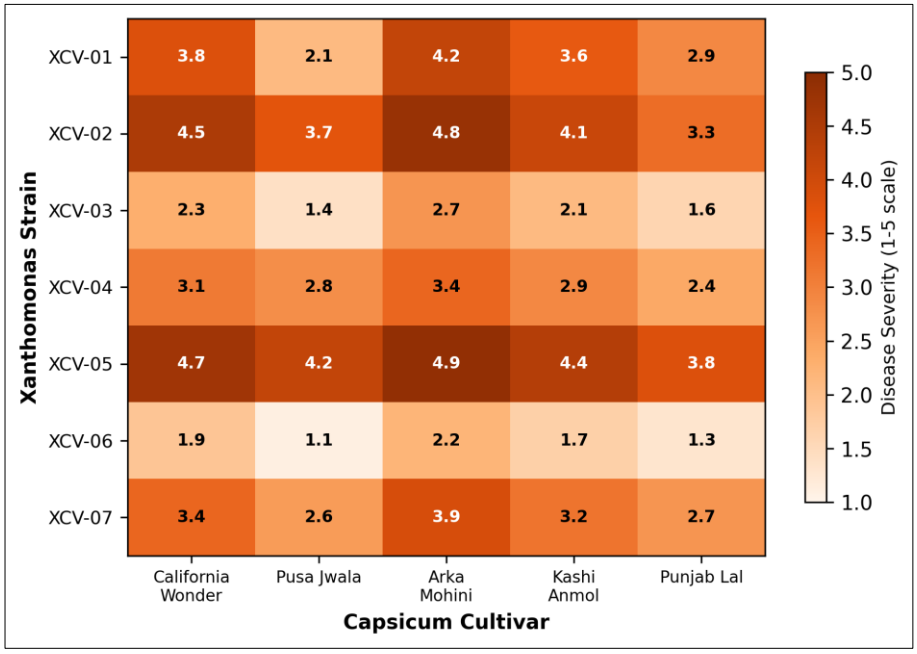

Fig 1: Heatmap of disease severity (1–5 scale) of seven *Xanthomonas euvesicatoria* strains on five capsicum cultivars at 14 days post-inoculation

Table 2: Antibiotic sensitivity of *Xanthomonas euvesicatoria* strains (inhibition zone diameter, mm)

Isolate	Strep. 100 ppm	Strep. 500 ppm	Cu-oxy 0.2%	Cu-oxy 0.3%	Kasug. 200 ppm	Control
XCV-01	14.3	24.7	8.1	11.4	16.2	0.0
XCV-02	12.1	22.3	7.6	10.8	14.7	0.0
XCV-05	11.8	21.9	6.9	9.7	13.8	0.0
Mean	13.1	23.4	7.8	10.9	15.3	0.0
CD (p=0.05)	1.4	1.9	0.8	1.1	1.3	

Strep. = streptomycin sulphate; Cu-oxy = copper oxychloride; Kasug. = kasugamycin. Representative strains shown.

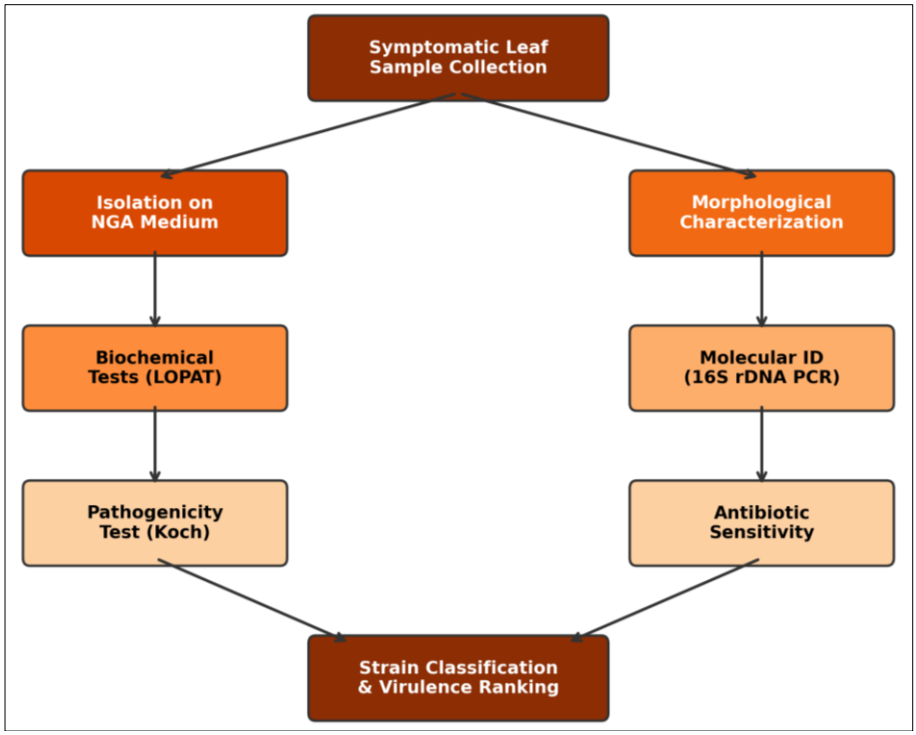

Fig 2: Workflow for isolation, identification, and virulence characterization of Xanthomonas strains from capsicum

Comprehensive Interpretation

All seven isolates belonged to *X. euvesicatoria* (≥98.7% 16S identity), indicating low species diversity but notable virulence variation within a single species. Strains XCV-02 and XCV-05 were highly aggressive on California Wonder and Arka Mohini (scores 4.5–4.9), whereas XCV-06 was the least virulent (maximum 2.2). Pusa Jwala showed the lowest disease scores across all strains, suggesting partial resistance. Streptomycin at 500 ppm produced the largest inhibition zones (mean 23.4 mm), followed by kasugamycin (15.3 mm), while copper oxychloride at 0.2% was the weakest (7.8 mm).

Discussion

The exclusive recovery of *X. euvesicatoria* in this survey contrasts with reports from Brazil and the United States where mixed populations of *X. perforans* and *X. euvesicatoria* co-occur [3, 9]. A possible explanation is that Gujarat's hot, semi-arid climate favours *X. euvesicatoria*, which has a higher thermal optimum than *X. gardneri* [4]. Broader surveys covering more districts and seasons would be needed to confirm whether other species are genuinely absent. The virulence spread (scores 1.1–4.9) within a single species has practical implications: resistance genes effective against low-virulence strains may fail when highly aggressive strains like XCV-05 dominate. Multi-strain screening of breeding lines is therefore necessary [10, 11].

Streptomycin's strong in-vitro efficacy is consistent with published data [12], but field application faces regulatory restrictions in several Indian states. Kasugamycin offers a viable alternative with lower environmental persistence [13]. Copper-based products alone appear insufficient against aggressive strains, though tank-mixing copper with a systemic antibiotic may improve results [14].

Conclusion

All seven *Xanthomonas* isolates from capsicum fields in Gujarat were identified as *X. euvesicatoria*, but their virulence differed markedly. Strains XCV-02 and XCV-05 were the most aggressive, while Pusa Jwala showed the highest field resistance across strains. Streptomycin sulphate at 500 ppm was the most effective antibiotic *in vitro*. These results provide a strain-level baseline for targeted resistance breeding and informed chemical management of BLS in western India.

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