World Journal of Research in All Subject
www.multiworldjournal.com
Online ISSN: 3107-8664
Received: 20-07-2026, Accepted: 19-08-2026, Published: 03-09-2026
Volume 2, Issue 3, 2026, Page No. 48-52

Awareness about citrus canker *(Xanthomonas axonopodis* pv. *Citri* isolates) in acid lime

Trilok Nath Rai[1], Kedar Nath Rai[2], Sanjeev Kumar Rai[3], Rajeev Kumar Rai[4], Pankaj Nautiyal[5]
[1] Department of Soil Science and Agricultural Chemistry, Krishi Vigyan Kendra (ICAR-CSSRI), Dhikunni, Sandila, Hardoi, Uttar Pradesh, India
[2] Associate Professor and Head, Department of Soil Science & Agricultural Chemistry, Kamla Nehru Institute of Physical and Social Sciences, Sultanpur, Uttar Pradesh, India
[3] Department of Soil Science & Agricultural Chemistry, Kamla Nehru Institute of Physical and Social Sciences, Sultanpur, Uttar Pradesh, India
[4] Department of Nursing, Institue of Nursing & Paramedical Sciences Sultanpur, Uttar Pradesh, India
[5] Head, Krishi Vigyan Kendra (ICAR-CSSRI), Dhikunni, Sandila, Hardoi, Uttar Pradesh, India

Abstract
Citrus canker, caused by *Xanthomonas citri* subsp. *citri*, is one of the most destructive bacterial diseases affecting acid lime cultivation. The present trials were undertaken to evaluate the pathogenicity of four isolates of *Xanthomonas axonopodis* pv. *citri* (Xac1, Xac2, Xac3 and Xac4) on different plant parts of acid lime (*Citrus aurantifolia* Swingle) - namely leaves, stems, fruits and thorns - and to assess pathogenic variability among isolates using an attached leaf technique under screenhouse conditions. The inoculation resulted in characteristic citrus canker symptoms including watery translucent spots, corky raised lesions and yellow halos. Leaves were found to be the most susceptible plant part, exhibiting the shortest incubation period across all isolates. Among the four isolates tested, Xac2 proved to be the most virulent, producing symptoms earliest (4 days on leaves) with the shortest average incubation period, while Xac4 was the least aggressive. Significant pathogenic variability was observed among the *X. citri* subsp. *citri* isolates, highlighting the importance of selecting virulent isolates for resistance screening and disease management strategies.

Keywords: Acid lime, citrus canker, *Xanthomonas citri* subsp. *Citri*, pathogenicity; pathogenic variability, virulence

Introduction

Acid lime (*Citrus aurantifolia* Swingle) is one of the most important citrus fruit crops cultivated in tropical and subtropical regions of the world. In India, it occupies a significant position owing to its wide use in the food, beverage, pharmaceutical and cosmetic industries. However, its cultivation is severely constrained by numerous biotic stresses, of which citrus canker is a major bacterial disease of economic importance. Citrus canker is caused by *Xanthomonas axonopodis* pv. *citri*, a Gram-negative, rod-shaped bacterium belonging to the family *Lysobacteraceae*. The pathogen is currently recognized as *Xanthomonas citri* subsp. *citri* (Xcc) based on reclassification.

The pathogen infects young and actively growing tissues, including leaves, twigs, thorns and fruits, resulting in characteristic raised, corky lesions surrounded by chlorotic halos. Severe infections lead to premature defoliation, twig dieback, blemished fruits, fruit drop, reduced marketability and considerable economic losses. The disease is widely distributed in major citrus-growing regions and spreads rapidly under warm temperatures, high humidity and wind-driven rain. The pathogenicity of *Xanthomonas axonopodis* pv. *citri* is influenced by both host susceptibility and pathogen aggressiveness. Different plant organs vary in their susceptibility owing to differences in tissue age, anatomical structure, stomatal density and physiological characteristics, which influence bacterial entry, colonization and symptom development. Consequently, evaluating pathogen infection on different plant parts is essential for understanding disease establishment and selecting reliable tissues for pathogenicity assays and disease resistance studies. Considerable variation in virulence has been reported among isolates of *Xanthomonas axonopodis* pv. *citri* collected from different geographical locations and citrus hosts. Such pathogenic variability may arise from genetic diversity, adaptation to local environmental conditions and differences in the expression of virulence-associated factors.

Variability among pathogen isolates can influence disease severity, host-pathogen interactions, epidemiological behaviour and the effectiveness of disease management strategies. Therefore, characterization of pathogenic variability is important for identifying highly virulent isolates, understanding pathogen diversity and developing durable disease management approaches, including host resistance screening. Although several studies have investigated citrus canker on different citrus species, comprehensive information regarding the comparative pathogenicity of *Xanthomonas axonopodis* pv. *citri* isolates on different plant parts of acid lime and the extent of pathogenic variability among isolates remains limited. Such information is crucial for standardizing artificial inoculation techniques, selecting representative virulent isolates for pathogenicity and screening experiments and improving our understanding of disease development in acid lime.

Therefore, the present investigation was undertaken to (i) evaluate the comparative pathogenicity of *Xanthomonas axonopodis* pv. *citri* on different plant parts of acid lime (*Citrus aurantifolia*), including leaves, twigs, thorns and fruits and (ii) assess the pathogenic (virulence) variability among different isolates based on symptom expression, incubation period, lesion development and disease severity.

Materials and Methods

1. Collection and Maintenance of Isolates

The acid lime plant samples (fruits, branches and leaves) showing typical symptoms of bacterial canker were collected in paper bags after surveying from 2022 to 2026 from various orchards, markets and kitchen gardens in the Sultanpur/ Hardoi, U.P. locality and brought to the Scientist & Associate professor. The diseased specimens were first subjected to an ooze test to confirm the association of the bacterium and then subjected to isolation on nutrient agar (NA) medium using standard procedures. Upon completion of the incubation period, single bacterial colonies developing on NA were picked aseptically, transferred to fresh NA Petri plates and incubated at 28 ± 2°C to obtain pure cultures of the bacterial isolates. The pure cultures thus obtained were preserved in a refrigerator for further studies.

2. Preparation of Bacterial Inoculum

A single bacterial colony from each test isolate cultured on nutrient agar medium was inoculated into NA broth for mass multiplication. The resultant cultures were kept at 28°C and shaken overnight at 200 rpm. The cultures were then collected and resuspended in sterile distilled water to a final concentration of 10^8 cfu/ml before inoculation. This cultured broth was used for all inoculation experiments.

3. Pathogenicity Test on Different Plant Parts and Assessment of Pathogenic Variability

Immature acid lime leaves (approximately 75% expanded) from healthy greenhouse-grown seedlings were selected for the pathogenicity assay. Leaf inoculation was carried out by the infiltration method using a sterile microneedle syringe. A freshly prepared bacterial suspension, adjusted to the desired concentration, was gently infiltrated through the abaxial (lower) surface of the leaf. Infiltration was continued until a water-soaked area of approximately 6 mm in diameter was produced. Three infiltration sites were made on each side of the midrib without overlapping, resulting in six inoculation sites per leaf. Three fully expanded immature leaves per plant were inoculated similarly. Immediately after inoculation, the inoculated shoots were enclosed in transparent polythene bags for 24 h to maintain high relative humidity, thereby providing favourable conditions for bacterial establishment and infection. The bags were subsequently removed and the plants were maintained under greenhouse conditions.

The inoculated leaves were examined periodically for symptom initiation and disease development and observations on the incubation period, lesion development and symptom progression were recorded up to 20 days after inoculation (DAI). The pathogenic variability of the four isolates (Xac1, Xac2, Xac3 and Xac4) of *Xanthomonas axonopodis* pv. *citri* was studied by the attached leaf technique under screenhouse conditions on leaves of the susceptible variety of acid lime (Sai Sharbati). Parameters recorded included incubation period (days), size of spots (mm) and number of spots per leaf. All experiments were conducted with three replicates per treatment.

Citrus canker is a contagious bacterial disease affecting lime and other citrus trees, caused by *Xanthomonas citri*. It creates raised, brown, corky lesions with yellow halos on leaves, stems, and fruit. While safe to eat, infected fruit is unmarketable, and severe cases cause premature leaf and fruit drop.

Symptoms

- Raised, rough, brown spots on fruit, leaves, and twigs.
- Water-soaked or oily margins surrounding the lesions.
- Yellow halos around young spots, which may turn corky and fade on older lesions.
- Defoliation, twig dieback, and early fruit drop during heavy infections.

Causes and Spread

- Caused by the bacterium *Xanthomonas citri*, which enters through natural pores (stomata) or physical wounds.
- Spread rapidly via wind-driven rain, water splashes, contaminated tools, and movement of infected plant material.
- Pests like the citrus leaf miner worsen the spread by creating feeding wounds that invite bacteria.

Management and Control

- No known cure exists once a tree is infected; prevention is critical.
- Prune and destroy heavily infected plant parts.
- Apply protective copper-based sprays or fixed copper bactericides (such as Bordeaux mixture) during flushing and wet seasons.
- Plant resistant citrus varieties where available and sterilize pruning tools between cuts.

Survey/ awareness/ trainings works done during 2009 to 2026 in the district of Bhadohi, Kushinagar, Sultanpur and Hardoi for identification and control measures of citrus canker in UP.

Results and Discussion

1. Pathogenicity and Comparative Analysis on Different Plant Parts

Pathogenicity of all four isolates of *Xanthomonas axonopodis* pv. *citri* causing bacterial canker of acid lime was confirmed by artificial inoculation under screenhouse conditions. All test isolates induced characteristic symptoms - small, watery, translucent yellow spots that progressed to become greyish and spongy with a rough corky surface, coalescing to form elongated lesions surrounded by a yellowish-brown raised margin and a yellow halo. Following symptom expression, re-isolation of the pathogen yielded cultures morphologically similar to the original isolates, thereby fulfilling Koch's postulates and confirming the causal role of the inoculated pathogen. Observations were made regularly for appearance and development of symptoms up to 20 DAI. Results regarding incubation period and symptomatology on different plant parts are presented in Tables 1-4.

The incubation period of Xac1 ranged from 9 days (leaves) to 20 days (thorn). The minimum incubation period was observed on leaves (9 days), followed by stem (13 days) and fruit (19 days). No differentiating symptoms were observed on thorns even after 20 days. The incubation period of Xac2 ranged from 4 days (leaves) to 8 days (fruit). The minimum incubation period was observed on leaves (4 days), followed by stem and thorn (7 days each). The incubation period of Xac3 ranged from 14 days (leaves) to 20 days; no

differentiating symptoms were observed on thorns or fruits even after 20 days. The incubation period of Xac4 was 15 days on leaves; no differentiating symptoms were observed on thorns, fruits, or stem within 20 days.

From the results, it is inferred that pathogenicity of all isolates varied on different plant parts. Xac2 was found to be the most virulent isolate, as evidenced by the earliest production of differentiating symptoms. This may be attributed to differences in pathogen genotype and virulence factor expression.

Table 1: Pathogenicity of Xac1 on different parts of acid lime plant

S. no.	Plant part	Incubation period (days)	Symptomatology
1.	Leaves	9-11	Watery translucent yellow spot
2.	Fruit	19-21	Watery yellow translucent yellow spot with brown margin
3.	Stem	13-15	Tiny corky spot
4.	Thorn	20-22	No differentiating symptoms were observed

Table 2: Pathogenicity of Xac2 on different parts of acid lime plant

S. no.	Plant part	Incubation period (days)	Symptomatology
1.	Leaves	4-5	Brown hard lesions with yellow halo
2.	Fruit	8-9	Corky tiny lesions
3.	Stem	7-8	Corky lesions
4.	Thorn	7-8	Black hard lesions

Table 3: Pathogenicity of Xac3 on different parts of acid lime plant

S. no.	Plant part	Incubation period (days)	Symptomatology
1.	Leaves	14-16	Watery translucent yellow lesion
2.	Fruit	20-22	No symptoms were observed
3.	Stem	19-21	Brown lesions
4.	Thorn	20-22	No symptoms were observed

Table 4: Pathogenicity of Xac4 on different parts of acid lime plant

S. no.	Plant part	Incubation period (days)	Symptomatology
1.	Leaves	15-17	Watery translucent tiny yellow lesions
2.	Fruit	20-22	No symptoms were observed
3.	Stem	20-22	No symptoms were observed
4.	Thorn	20-22	No symptoms were observed

2. Pathogenic Variability among Isolates

The results (Tables 5, 6 and 7) revealed that all test isolates of *X. axonopodis* pv. *citri* was pathogenic and caused bacterial canker in acid lime (cv. Sai Sharbati). On leaves inoculated with Xac1, initial symptoms appeared as watery translucent spots with yellow halo on the 8th day; on the 12th day, spots exhibited a greyish and spongy center; whereas on the 16th and 20th day, rough corky spots with raised brown margin were observed. Xac2 produced initial symptoms as watery translucent spots with yellow halo on the 4th day; on the 12th day, spots showed a yellow halo; and on the 16th and 20th day they became rough corky with raised brown margin. Xac3 showed initial symptoms as watery translucent spots on the 12th day, similar on the 16th day, becoming rough corky spots with raised brown margin on the 20th day. Xac4 showed initial symptoms as watery translucent spots on the 12th day, watery translucent spots with yellow halo on the 16th day and rough corky spots with raised brown margin on the 20th day.

The present investigation confirmed the pathogenicity of all four isolates of *Xanthomonas citri* subsp. *citri* on acid lime through artificial inoculation under screenhouse conditions. The development of characteristic citrus canker symptoms and successful re-isolation of the bacterium from infected tissues fulfilled Koch's postulates. Similar symptom development and pathogenicity confirmation have been reported by Schubert *et al*. (2001), Graham *et al*. (2004) and Cubero and Graham (2005) [4, 6, 8], who described the typical progression of citrus canker lesions from water-soaked spots to raised corky pustules surrounded by yellow halos following artificial inoculation.

The results further demonstrated significant differences in pathogenic behaviour among the isolates on different plant organs. The incubation period varied considerably depending on both the bacterial isolate and the host tissue inoculated. Among all the isolates, Xac2 produced symptoms earliest on leaves (4 days), stem and thorn (7 days) and fruits (8 days), whereas Xac3 and Xac4 required considerably longer incubation periods and Xac4 failed to produce visible symptoms on stem, thorn and fruit within the observation period. Such differences indicate the existence of pathogenic variability among the isolates of *X. citri* subsp. *citri*. Similar variability in aggressiveness among isolates has been reported by Vernière *et al*. (1998), Cubero and Graham (2002) and Bui Thi Ngoc *et al*. (2010) [2, 3, 9], who observed that isolates of the citrus canker pathogen differ significantly in their ability to infect citrus tissues and in the rate of symptom development.

The superior virulence exhibited by Xac2 is particularly noteworthy. Although isolated from infected fruits, Xac2 exhibited the shortest incubation period and rapid progression of disease symptoms. Citrus fruits contain relatively high concentrations of antimicrobial secondary metabolites, including phenolic compounds, flavonoids and essential oils, which generally restrict pathogen establishment (Lattanzio *et al*., 2006) [7]. The ability of Xac2 to establish infection rapidly despite originating from such a chemically defended tissue suggests that this isolate

possesses enhanced virulence characteristics, possibly resulting from more efficient detoxification of host antimicrobial compounds, higher bacterial multiplication rates, or stronger expression of Type III secretion system effectors (Brunings and Gabriel, 2003; da Silva *et al*., 2002) [1, 5].

The observed pathogenic variability among isolates may also be attributed to differences in the production of extracellular polysaccharides (EPS), hydrolytic enzymes, biofilm formation and other pathogenicity determinants. Genomic studies have demonstrated considerable variation in virulence-associated genes among *X. citri* isolates, leading to differences in symptom expression and aggressiveness (da Silva *et al*., 2002) [5]. Moreover, repeated adaptation of bacterial populations to different host organs may select for isolates possessing distinct pathogenic capabilities, thereby contributing to the observed variability. The variation in incubation period, lesion frequency and lesion size observed in the present study may also reflect differences in host defence responses. Citrus tissues produce constitutive antimicrobial compounds (phytoanticipins) and inducible defence metabolites (phytoalexins) following pathogen attack. Isolates capable of overcoming or delaying these defence mechanisms establish infection more rapidly and produce more severe symptoms (Lattanzio *et al*., 2006) [7].

Overall, the present findings confirm the existence of significant pathogenic variability among *X. citri* subsp. *citri* isolates infecting acid lime. Although all isolates were pathogenic, Xac2 was the most virulent due to its significantly shorter incubation period and rapid symptom development, whereas Xac3 exhibited greater lesion expansion. These differences emphasize the importance of considering isolate variability during resistance screening, epidemiological studies and the development of integrated management strategies for citrus canker.

Table 5: Pathogenic variability among the Xac isolates (Incubation period)

S. no.	Isolates	Incubation period (days)	Incubation period (days)	Incubation period (days)	Incubation period (days)	Incubation period (days)	Incubation period (days)
	Isolates	4th	8th	12th	16th	20th	Average of 20 days*
1.	Xac1	No symptoms were observed	Watery translucent spot with yellow halo	Spots with greyish and spongy center	Rough corky spots with raised brown margin	Rough corky spots with raised brown margin	9.66
2.	Xac2	Watery translucent spot	Watery translucent spot	Watery translucent spot with yellow halo	Rough corky spots with raised brown margin	Rough corky spots with raised brown margin	14.66
3.	Xac3	No symptoms were observed	No symptoms were observed	Watery translucent spot	Watery translucent spot	Rough corky spots with raised brown margin	19.33
4.	Xac4	No symptoms were observed	No symptoms were observed	Watery translucent spot	Watery translucent spot with yellow halo	Rough corky spots with raised brown margin	20.33
*Indicates mean of three replicates	*Indicates mean of three replicates	*Indicates mean of three replicates	*Indicates mean of three replicates	*Indicates mean of three replicates	*Indicates mean of three replicates	*Indicates mean of three replicates	*Indicates mean of three replicates

Table 6: Pathogenic variability among the Xac isolates (Size of the spot)

S. no.	Isolates	Size of spots*(mm)	Size of spots*(mm)	Size of spots*(mm)	Size of spots*(mm)	Size of spots*(mm)	Size of spots*(mm)
	Isolates	4th day	8th day	12th day	16th day	20th day	Average of 20 days
1.	Xac1	0.00	0.10	0.50	1.00	1.20	1.50
2.	Xac2	0.25	0.90	2.45	4.92	5.00	5.33
3.	Xac3	0.00	0.25	1.95	5.62	5.68	6.00
4.	Xac4	0.00	0.00	0.25	1.45	2.64	3.16
*Indicates mean of three replicates	*Indicates mean of three replicates	*Indicates mean of three replicates	*Indicates mean of three replicates	*Indicates mean of three replicates	*Indicates mean of three replicates	*Indicates mean of three replicates	*Indicates mean of three replicates

Table 7: Pathogenic variability among the Xac isolates (Number of spots)

S.no.	Isolates	No. of spots*	No. of spots*	No. of spots*	No. of spots*	No. of spots*	No. of spots*
	Isolates	4th day	8th day	12th day	16th day	20th day	Average of 20 days
1.	Xac1	0.00	1.00	1.00	3.00	4.00	4.00
2.	Xac2	1.00	1.25	1.25	1.30	1.50	1.33
3.	Xac3	0.00	0.00	1.50	1.55	1.60	1.66
4.	Xac4	0.00	0.00	0.00	1.25	1.30	1.33
*Indicates mean of three replicates	*Indicates mean of three replicates	*Indicates mean of three replicates	*Indicates mean of three replicates	*Indicates mean of three replicates	*Indicates mean of three replicates	*Indicates mean of three replicates	*Indicates mean of three replicates

Summary and Conclusion

This, trials confirmed the pathogenicity of four isolates of *Xanthomonas citri* subsp. *citri* on acid lime and demonstrated clear differences in their virulence. All the isolates produced characteristic citrus canker symptoms and fulfilled Koch's postulates. Among the different plant parts

tested, young leaves were the most susceptible, showing the shortest incubation period and rapid symptom development, while stems, fruits and thorns were comparatively less susceptible. Significant pathogenic variability was observed among the isolates. Xac2 was identified as the most virulent isolate because of its early symptom expression and shorter incubation period, whereas Xac3 produced the largest lesions and Xac1 developed the highest lesion frequency. In contrast, Xac4 was comparatively less aggressive. Overall, the study highlights the existence of pathogenic variability among *X. citri* subsp. *citri* isolates infecting acid lime. These findings will be useful for selecting virulent isolates for resistance screening, understanding disease epidemiology and developing effective and sustainable management strategies for citrus canker.

Declaration of AI Use

This manuscript was prepared through the combined contributions of all author(s), including contributions to the study design, data, content development, results, interpretation, and related scholarly work. The author(s) acknowledge the use of Grammarly and ChatGPT to assist with grammar checking, language refinement, reference formatting. These AI-assisted tools were not used as authors and did not replace the intellectual contributions or scholarly judgment of the author(s). All AI-assisted outputs, including content, references, and interpretations, were carefully reviewed, revised, verified, and approved by the author(s). The author(s) accept full responsibility for the accuracy, integrity, and final content of the manuscript.

Acknowledgements

Author Dr. Trilok Nath Rai thankful to corresponding author Dr. Kedar Nath Rai, Associate Professor and Head, Department of Soil Science & Agricultural Chemistry, Kamla Nehru Institute of Physical and Social Sciences, Sultanpur, Uttar Pradesh, India for preparation of manuscript & financial supports for publications

Citrus canker *(Xanthomonas axonopodis* pv. *citri* Isolates) in acid lime

References

1. Brunings AM, Gabriel DW. Xanthomonas *citri*: breaking the surface. Molecular Plant Pathology,2003:4:141–157.
2. Bui Thi Ngoc L, Vernière C, Jouen E, Ah-You N, Lefeuvre P, Chiroleu F, *et al*. Diversity of Xanthomonas *citri* subsp. *citri* populations associated with citrus canker. Plant Pathology,2010:59:270–280.
3. Cubero J, Graham JH. Genetic relationship among worldwide strains of Xanthomonas causing canker in citrus species and design of new primers for their identification by PCR. Applied and Environmental Microbiology,2002:68:1257–1264.
4. Cubero J, Graham JH. Quantitative PCR method for diagnosis of citrus bacterial canker. Applied and Environmental Microbiology,2005:71:8740–8748.
5. da Silva ACR, *et al*. Comparison of the genomes of two Xanthomonas pathogens with differing host specificities. Nature,2002:417:459–463.
6. Graham JH, Gottwald TR, Cubero J, Achor DS. *Xanthomonas axonopodis* pv. *citri*: factors affecting successful eradication of citrus canker. Molecular Plant Pathology,2004:5:1–15.
7. Lattanzio V, Lattanzio VMT, Cardinali A. Role of phenolics in the resistance mechanisms of plants against fungal pathogens and insects. In Phytochemistry: Advances in Research,2006:23–67.
8. Schubert TS, Rizvi SA, Sun X, Gottwald TR, Graham JH, Dixon WN. Meeting the challenge of eradicating citrus canker in Florida. Plant Disease,2001:85:340–356.
9. Vernière C, Hartung JS, Pruvost OP, Civerolo EL, Alvarez AM, Maestri P, *et al*. Characterization of phenotypically distinct strains of *Xanthomonas axonopodis* pv. *citri* from Southwest Asia. European Journal of Plant Pathology,1998:104:477–487.