Eco. Env. & Cons. 29 (January Suppl. Issue) : 2023; pp. (S333-S337)
Copyright@ EM International
ISSN 0971–765X

DOI No.: http://doi.org/10.53550/EEC.2023.v29i01s.050

Prevalence of Citrus Canker caused by *Xanthomonas axonopodis pv.citri.* in Eastern and Northern region of Madhya Pradesh, India

Prashant Singh[1], Prashant Kumar Singh[1]*, Shyam Kishor Patel[1], Sukram Thapa[1] and N. Shivajyothi[1]

[1]Department of Plant Pathology, School of Agriculture, ITM University, Gwalior, M.P., India

(Received 11 June, 2022; Accepted 10 August, 2022)

ABSTRACT

Bacterial canker is one of the most common citrus diseases, causing widespread losses throughout the citrus growing regions of the world, including India. Madhya Pradesh is one among the leading states in India for citrus production. Therefore, to Keep track of the disease prevalence on lime, lemon, malta, grapefruit, and other citrus fruits, a survey was conducted in eastern (Baghelkhand) and northern (Gird) regions of Madhya Pradesh during the months of February to March 2022. Various crucial data were collected on disease severity (percentage) and disease incidence (percentage). During the survey, disease incidence was recorded from 0% to 40% range, and disease severity from 0% to 37%, with a maximum mean disease incidence (36.5%) and severity (34%), respectively, in Chinor tahsil of Gwalior district and a minimum disease incidence (18%) and severity (17.3%) in Satna district of Madhya Pradesh. Disease samples of *Xanthomonas axonopodis pv.citri* were collected from various villages in the chosen district during the survey (Turari, Bhanduli, Khohar, Ambha, and Panihar). After three days of purification, all five isolates showed a light yellow to yellow colour, a mucoid surface, and a circular colony with a diameter of 4 to 5.5 mm. The biochemical characteristics of bacterial isolates subjected to various biochemical tests, including the catalase test, KOH solubility test, H_2S production, and Gram reaction, were studied. All isolates were positive in catalase, KOH solubility, and H_2S production tests, but negative in Gram's reaction. The virulence behaviour of the isolates, namely *Xacl, Xac2, Xac3, Xac4*, and *Xac5*, was diagnosed during the pathogenicity test by spraying the isolate suspension on the citrus plant. Symptoms appear 12-17 days after inoculation with a different isolate. Lesions appear on the leaf's bottom surface as small, translucent, round spots. A yellow halo surrounded the lesions. Isolate *Xac3* develops water-soaked lesions on the leaves 12 days after inoculation. Other isolates, such as *Xac4*, had low virulence, with symptoms showing up after16days.*Xac1, Xac2*, and *Xac5* were all partially virulent isolates.

Key words: *Citrus canker, Xanthomonas axonopodis.*

Introduction

Citrus (*Citrus spp.)* belongs to the Rutaceae family and the Aurantioedae subfamily (Swingle 1943). This is a very important fruit crop of India, after mangoes and bananas, lime encompasses 1.07 million ha. area, yielding 10.49 million tonnes total and 9.77 tonnes/ha average in 2017. The leading lime producing states in India are Andhra Pradesh, Madhya Pradesh, Panjab, Maharastra, and Gujarat (Horticulture website, 2013). Madhya Pradesh is the country's fourth largest producer of citrus fruits, accounting for 10.8 percent of total production, with a production of 0.80 MT of citrus on an area of 0.05 million hectare. Average state production is 17.7 tons/ha. Chindawara, Mandasaur, Khandwa, and

Hoshangabad are the major citrus producing districts (MIDH, 2013). Lime contains a lot of nutritious and refreshing food, as well as flavour and aroma. This is high in vitamins B1, B2, folate and C (62.mg/100 ml), minerals like phosphorus (20 mg/100ml), calcium (90mg/100 ml), magnesium (8 mg/100 ml), potassium (138 mg/100 ml amino acids, sugars, and other nutrients (Sarolia and Mukherjee, 2002). Many agricultural pests and diseases that threaten citrus plants are caused by fungi, viruses, and bacteria. Bacterial inflamation is lime canker caused by (*X.axonopodispv.citri Hasse*). Citrus canker is the most damaging citrus disease in the world (Gottwald, 2002). Citrus bacterial spot is thought to have introduced in India or Southeast Asia and has since dispersed throughout the world (Civerolo 1984; Vernière *et al.*, 1998). It was discovered in 1915 in Florida (USA). Hasse (1915) was the first to isolate organisms and prove pathogenicity. Citrus canker is caused by a variety of pathovars and variants of the bacterium *Xanthomonas axonopodis* (Graham *et al.*, 1990). Most Citrus cultivars are susceptible to bacterial canker, therefore, to restrict further dissemination, mass elimination of infected plants began in the southern states of the United States in 1915, and the disease was confirmed put an end on 1947. Subsequent epidemics were reported in over 30 countries in Asia, New Zealand, Africa, and South Africa; it reappeared in the 1980s and was reported to be United States, Africa and Australia. Afterward, cases were reported in Mexico in 1981 and Florida in 1984, which appear to be distinct from in Asia (Goto, 1992). In 1995, a novel and widespread outbreak was found in urban Miami, Florida (Schubert and Miller, 1996). Fruit canker was first discovered in India in Punjab (Luthra and Sattar, 1942). Several research viz, Bal and Dhiman (2005) identified citrus canker (*Xanthomonus axonopodis pv. citri*) in Kinnow mandarin nursery and studied the relationship between disease development and environmental factors. The highest incidence of citrus canker (73.3%) was recorded in the second week of September. Talib Sahi *et al.* (2007) in three tehsils (Pakistan), the incidence of citrus canker disease were7.5 percent.

Materials and Methods

Survey and collection of disease sample in Baghelkhand and Gird region

A survey was conducted in four districts of Madhyap Pradesh, i.e., Gwalior, Morena, Satna and Rewa to assess the incidence and severity of citrus canker. The disease's incidence and severity on fruit and leaf were recorded. Nearly 100-150 leaves were collected from five randomly selected plants, showing typical canker symptoms.Using the standard scale developed by Seif and Hillocks (1999), disease incidence on leaf (Wheeler, 1969), and disease severity were calculated using the given formula.

$$\text{Disease incidence} = \frac{\text{Total number of infected plant}}{\text{Total number of plant observed}} \times 100$$

$$\text{Percent disease severity} = \frac{\text{Number of diseased leaves}}{\text{Total number of leaf observed}} \times 100$$

During the survey, the Canker-infected lime plant fruit and leaf samples were collected from an orchard at ITM University Gwalior and surveyed villages (Turari, Bhanduli, Khohar, Ambha and Panihar) then placed in plastic bags, transported to the lab, and stored for microscopic analysis.

Isolation of the pathogen

Small bits of tissue measuring 4-5 mm were taken from the infected leaf, fruitor stem, cut with a sterilized blade, surface sterilised with Sodium hypochlorite 0.1 percent for 10-15 seconds, and then cleaned in distilled water. To allow the bacteria to ooze out of the infected portion, the cut slices were placed in distilled water for 5 minutes. Under the cabinet of laminar air flow, a loopful of this inoculum was streaked on already poured NA petriplates. The inoculated plates were incubated in the BOD incubator for 24 to 48 hours in the inverted position at 30 °C.

Identification of the pathogen

To identify the pathogen by the morphological, cultural and biochemical characters.

Morphological and Cultural characters

Five 250 ml conical flasks were filled with 100 ml nutrient broth media and sterilized for 15 minutes at 121 °C and 15 lb pressure. After cooling at room temperature, each isolated bacterial colony was inoculated in the conical flask and incubated at 28±1°C for 24 to 48 hours. After incubation, 1 ml of each flask's bacterial suspension was taken and serially diluted up to a dilution factor of 10^{-5}cfu and were inoculated in Nutrient Agar Media (NAM) poured petriplates. These plates were incubated for 48 to 72 hours at 28±1°C in the BOD incubator. Color, mar-

gin, size, shape, surface, and elevation of each colony were recorded for each isolate.

Biochemical characters

The biochemical attributes of bacterial isolates subjected to various biochemical tests, such as the catalase test, KOH solubility test, H_2S production and gram reaction, were investigated. To confirm and identify all isolates as per the methodology given by Bergey's Manual of Determinative Bacteriology (1923).

Pathogenicity test

The bacteria *Xanthomonas axonopodis pv.citri* was isolated from various plant parts which were collected from University orchard and various villages in the selected district (Turari, Bhanduli, Khohar, Ambha and Panihar). The isolates were then purified and kept separately on NAM slants. Using a spray inoculation technique, pathogenicity of Xac1, Xac2, Xac3, Xac4, and Xac5 isolates was tested on susceptible Citrus trees (Variety).

Results and Discussion

Survey and collection of disease sample in Baghelkhand and Gird region

The data presented (Table 1) clearly revealed that the maximum percent disease incidence was 36.5% in Chinor tahsil, followed by Dabara (35%), Mangawan (35%), Kotar (33.5%), Morena (30.5%), Jawa(29.5%), Gwalior (28.5%), Sirmaur (24%), Ambha (22%), Amarpatan (14%), and Nagod (12.5%). The minimum percent disease incidence of citrus canker of acid lime was found in Majhgawan, i.e., 12.0%. Teonthar had a disease incidence of citrus canker of acid lime of 0%. The Highest percent disease severity was found in Chinor tahsil (34%), followed by Kotar (33%), Mangawan (31.5%), Dabara (29%), and Morena (28%). The lowest percent disease severity was observed in Majhgawan (9%). Teonthar reported 0% disease severity. However, the maximum average percent disease incidence and severity were found in Gwalior district (33.3 and 28.8%), followed by Morena district (26.25 & 26%) then Rewa district (22 & 18.6%). Satna district had the lowest average percent disease incidence and severity (18 and 17.3%, respectively).

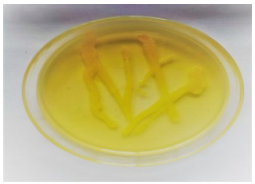
Isolation of pathogen

Pathogens were isolated and purified on NA media using bacterial ooze obtained from a canker-infected lime leaf. Bacterial colonies formed after 24 to 48 hours of incubation *Xanthomonas* colonies appeared yellow in color. Five isolates were obtained from leaf

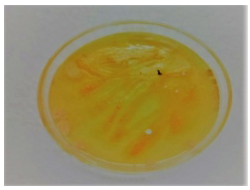
Table 1. Tahsil wise incidence and severity of citrus canker on acid lime in the orchard of Gwalior, Morena, Satna and Rewa districts.

Sr. No.	Districts	Tahsil		No. of village surveyed	Disease percent	
					Incidence	Severity
1.	Gwalior	Dabara		2	35	29
2.		Gwalior		2	28.5	23.5
3.		Chinor		2	36.5	34
			Total	6		
				Mean	33.3	28.8
4.	Morena	Ambha		2	22	24
5.		Morena		2	30.5	28
			Total	4		
				Mean	26.25	26
6.	Satna	Amarpatan		2	14	16.5
7.		Majhgawan		2	12	9
8.		Nagod		2	12.5	11
9.		Kotar		2	33.5	33
			Total	8		
				Mean	18	17.3
10.	Rewa	Teonthar		2	00	00
11.		Mangawan		2	35	31.5
12.		Sirmaur		2	24	19
13.		Jawa		2	29.5	24
			Total	8		
				Mean	22	18.6

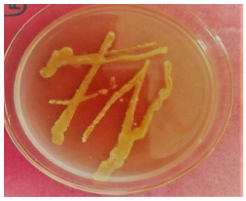
samples collected in various locations and named *Xac1*, *Xac2*, *Xac3*, *Xac4*, and *Xac5*.



Xac 1



Xac 2



Xac 3

Xac4

Xac5

Cultural characteristics of *X. auxonopodishpv.citri*

The cultural characterization of five isolate colonies of *X.campestris pv. citri* were investigated using Nutrient Agar Media (NAM) as a basal medium. Three isolates, *Xac2*, *3*, and *5*, were yellow, while the other two, *Xac1* and *4*, were yellow to light yellow in color. The colony's shapes and surfaces were all mucoid and circular.

Biochemical characteristics

Catalase test

When bacteria were combined with a few droplets of 3 percent H_2O_2 on a glass slide, gas bubbles formed. The catalase test was used to determine which organisms produced the enzyme. Hydrogen peroxide is detoxified by this enzyme, which breaks it down into water and oxygen gas. A catalase positive result is clearly attributed to the presence of bubbles generated by the production of O_2 gas.

KOH solubility test

The Gram reaction was confirmed when all of the test isolates reacted positively to the KOH solubility test. The appearance of slime strings indicates the existence of gram-negative bacteria, which have comparatively weak cell walls surrounded by an outer membrane. KOH instantly disrupts this, allowing the viscous DNA to escape. On the other hand, gram-positive bacteria have a thicker, more rigid cell wall that protects them from the harmful effects of KOH. The bacterium reacted positively to the KOH test, according to the present investigation.

Catalase test **H_2S production test** **KOH solubility test**

Hydrogen sulphide (H_2S) production

It was demonstrated that strains of *X. campestris pv. citri* generated the gas, which then reacted with the lead acetate strips, forming a precipitate at the strips' apex. As a result of the reaction, lead acetate-treated strips turned black. This reveals the existence of hydrogen sulphide (H_2S).

Pathogenicity test

The virulence behaviour of the bacteria/isolates, namely *Xacl*, *Xac2*, *Xac3*, *Xac4*, and *Xac5*, was diagnosed by spraying the isolate suspension on the citrus plant, as according Koch's postulate. In the evening, a 10^5cfu/ml cell suspension was sprayed with a hand sprayer.Symptoms appear 12–17 days after a different isolate is inoculated. Lesions begin

Table 2. The list of isolates of *X. axonopodis pv.citri* obtained from different places.

Sr.No.	Name of Isolate	Place of Isolate	Host	Affected plant part
1	*Xac1*	Turari village	*C. aurantifolia*	Leaf
2	*Xac2*	Bhanduli village	*C. aurantifolia*	Leaf
3	*Xac3*	Khohar village	*C. aurantifolia*	Leaf
4	*Xac4*	Deoravillage	*C. aurantifolia*	Leaf
5	*Xac5*	Panihar village	*C. aurantifolia*	Leaf

Table 3. Cultural characteristic of *X. campestris pv.citr i*isolates

Sr. No.	Isolates	Colony color	Colony shape	Margin	Elevation	Cell shape	After 72 hrs colony diameter (mm)
1.	Xac1	Light yellow	Circular	Entire	Convex	Rod	4.5
2.	Xac2	Yellow	Circular	Entire	Convex	Rod	4.8
3.	Xac3	Yellow	Circular	Entire	Convex	Rod	5.5
4.	Xac4	Light yellow	Circular	Entire	Convex	Rod	4
5.	Xac5	Yellow	Circular	Entire	Convex	Rod	5.1

as small, translucent, round spots on the leaf's bottom surface. There was a yellow halo around the lesions. Within 12 days after inoculation, isolate *Xac3* develops water-soaked lesions on the leaves. Other isolates, such as *Xac4,* had low virulence, with symptoms appearing after 16 days of infection and it shows disease incidence 18% and disease severity 14%. *Xac1, Xac2*, and *Xac5* were partially virulent isolates. The Isolate *Xac3* defines disease incidence 40% and disease severity 37% was found to be the most potent and virulent of the group, followed by the other isolates.*Xac1* shows disease incidence was 31% and disease severity 25 %, *Xac2*defines disease incidence rate 18% and severity 21%, *Xac5* shows disease incidence 26% and disease severity 22%.

Conclusion

Citrus canker was prevalent on sour limes in Madhya Pradesh. According to the findings of the survey, Gwalior district had the highest average percent disease incidence and severity (33.3 and 28.8%, respectively), while Satna district had the lowest average percent disease incidence and severity (18 and 17.3 percent, respectively). On the basis of biochemical tests such as KOH solubility, H_2S production, and catalase tests, the associated pathogen was identified as *X. axonopodis pv. citri.* Five bacterial isolates were collected (*Xac1, 2, 3, 4, 5*). The colors of *Xac2, 3,* and *5* were yellow, while the colors of Xac1 and 4 were yellow to light yellow. After inoculation, symptoms appear 12–17 days later. Within 12 days after inoculation, isolate *Xac3* develops water-soaked lesions on the leaves. Other isolates, such as *Xac4,* had low virulence, with symptoms appearing after 16 days of infection. *Xac1, Xac2*, and *Xac5* were partially virulent isolates.

References

Bal, R.S. and Dhiman, J.S. 2005. An analysis of synchronous progress of canker-scab complex in Kinnow nursery. *Plant Disease Research.* 20: 147-150.

Bergey's Manual of Determinative Bacteriology, 1957, Williams and Wilkins Co.in English - 7th ed.

Goto, M. 1992. Citrus Canker. In: *Plant Diseases of International Importance* Vol.III (Kumar, J., Chanbe, V, S., Singh, A. and Mukhopodhyay, A.N. Eds), Prentice-Hall, Englewood, Cliff, NJ. 170-208.

Gottwald, T.R., Graham, J.H. and Schubert, T.S. 2002. Citrus canker: the pathogen and its impact. *Plant Health Progress*. 3(1): 15.

Graham, J.H., Hartung, J.S., Stall, R.E. and Chase, A. R. 1990. Pathological, restriction-fragment length polymorphism, and fatty acid profile relationships between *Xanthomonas campestris* from citrus and noncitrus hosts. *Phytopathology*. 80(9): 829-836.

Hasse, C.H. 1915. *Pseudomonas citri*, the cause of citrus canker. *Journal of Agricultural Research*. 4(1): 97-104.

Luthra, J.C. and Sattar, A. 1942. Citrus canker and its control in Punjab. *Punjab Fruit J.* 6(1) : 179-182.

Sahi, S.T., Ghazanfar, M.U., Afzal, M., Rashed, A. and Habib, A. 2007. Incidence of citrus canker disease caused by *Xanthomonas campestris pv. citri (Hasse)* dows on Kinnow (*Citrus reticulata*) and its chemotherapy. *Pakistan Journal of Botany*. 39(4): 1319.

Sarolia, D.K. and Mukherjee, S. 2002. The efficacy of various preservation methods in preserving the quality of lime juice (*Citrus aurantifolia Swingle* (L.) during storage. *Haryana Journal of Horticultural Sciences*. 31 (3/4): 185-188.

Schubert, T.S. and Miller, J. W. 1996. *Bacterial citrus canker*. Fla. Department Agric. & consumer Services, Division of Plant Industry.

SEIF, A.A. and Hillocks, R. 1999. Reaction of some citrus cultivars to Phaeoramularia fruit and leaf spot in Kenya. *Fruits* (1978). 54(5): 323-329.

Swingle, W.T. 1943. The botany of citrus and its wild relatives of the orange subfamily (family Rutaceae, subfamily Aurantioideae) is well known.

Verniere, C. J., Gottwald, T. R. and Pruvost, O. 2003. Disease development and symptom expression of *Xanthomonas axonopodis pv. citri* in various citrus plant tissue *Phytopathology.* 93(7): 832-843.

Wheeler, B. E. J. 1969. An introduction to plant diseases. *An introduction to Plant Diseases.*