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Studies on different acid lime cultivars reaction to isolates of *Xanthomonas axonopodis* PV. *Citri*

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Abstract
In present research work Sixteen different isolates of *Xanthomonas axonopodis* pv. *citri* were tested on different acid lime cultivars. The pathogenicity was confirmed by reaction of these isolates on different citrus cultivars by inoculation. It was found that variety Chankradhar was most susceptible to all the isolates of *Xanthomonas axonopodis* pv. *citri* and expressed symptoms in 3 -10 days after inoculation. Xac1 and Xac12 produced symptoms on cultivar Chankradhar 3 days after inoculation followed by Xac 2, Xac11 and Xac16. However, only two Xac isolates **viz**. Xac12 and Xac 13 were produced symptoms on cultivar Bagalipattu and expressed symptoms within 4 day of inoculation. The isolate Xac12 and Xac14 represented distinct nature of virulence as it initiated water soaked lesion within three to six days. Tahiti was found resistant against all isolates as none of the isolate was able to cause the disease.

Keywords: Citrus, *Xanthomonas axonopodis* pv. *Citri*

Introduction
Citrus is an important fruit crop of the world. Present day citrus is delectable, juicy and seedless is of great nutritional significance as well (Khan *et al*., 1992). It is popular in both fresh and processed form. It is known for its high nutritive and refreshing value, taste, attractive fragrance. Citrus is a good source of vitamin C (62.9 mg/100 ml), B_1, B_2 and minerals like calcium (90 mg /100 ml), phosphorus (20 mg/100 ml) and iron (0.3 mg/100 ml), (Saloria and Mukherjee, 2002) [9]. Citrus canker is one of the most destructive and predominant on acid lime in India. Citrus bacterial canker (CBC), caused by *Xanthomonas citri* subsp *citri* (Schaad *et al*. 2006) [10] is one of the most devastating diseases through the world that affects many kind of commercial citrus varieties. It was first identified in Florida (USA) in 1915 and in India was reported from Punjab in 1942. The main symptoms of CBC are hyperplasia-type lesions on leaves, fruit and stems. In severe infections causes leaf abscission, twig dieback and premature fruit drop (Stall and Civerolo 1991; Gottwald *et al*. 1993) [11, 3]. The bacterium was first named as *Pseudomonas citri* (Hasse, 1915). In 1939 it was classified as genus *Xanthomonas* sp. (*X. citri*), then reclassified in 1980 (Dye *et al*., 1980) [2] as *Xanthomons campestris* pv. *citri* due to inadequate phenotypic data (Young *et al*., 1978) [13].
There are many types of citrus canker caused by various pathovars and variants of the bacterium *Xanthomonas axonopodis* (Graham *et al*., 1990) [4]. All cultivars of citrus are susceptible to canker, but grapefruit, Mexican lime and lemon are highly susceptible, whereas sour orange and sweet orange are moderately susceptible. Mandarins are moderately resistant (Gottwald *et al*., 1993) [3]. All young, above-ground tissues of citrus are susceptible to *Xanthomonas axonopodis*. In fact, bacterial pathogens infects into the plant tissues through natural openings (stomata) and mechanical injuries (wounds). *Xanthomonas axonopodis* pv. *citri* is a rod shaped gram negative bacterium with single polar flagellum. It is obligatorily aerobic, non spore former and produce yellow colonies on NA medium. The maximum temperature for growth is 35 to 39°C (Mehrotra, 1980; Whiteside *et al*., 1988) [7, 12]

Material and methods
Collection and isolation of diseased plant samples
Collection of diseased samples
Disease samples of Citrus canker were collected from the 14 agro-climatic regions of India (Table-1). The diseased samples of acid limes were colleted during the month of July to

October 2017. The fresh infected leaves sample were used for isolation by empolying tissue isolation method on NA medium.

Identification of the pathogen

The identification of the *Xanthomonas axonopodis* pv. *citri* was done as per available internals and by morphological, cultural and physiological features of the pathogen as per standard microbiological procedures.

Table 1: Details of disease samples collected from Agro climatic regions of India

No.	Agro climatic regions	State	Location	Designation	Plant part used for isolation
1	Western Plateau Hill Region	Akola (M.S.)	AICRP Dr.P.D.K.V. Akola	Xac-1	Leaf
2	Eastern Plateau Hill Region	Nagpur (M.S.)	Nagpur	Xac-2	Leaf
3	Western Plateau Hill Region	Pune (M.S.)	Pune	Xac-3	Leaf
4	West Cost Plane and Ghat Region	Rahuri (M.S.)	MPKV, Rahuri	Xac-4	Leaf
5	West Coast Plains and Ghat Region	Dapoli (M.S.)	Dapoli	Xac-5	Leaf
6	Southern Plateau Hill Region	Andhra Pradesh	Kurnool	Xac-6	Leaf
7	Upper Gangentic Plane	Uttar Pradesh	BHU, Varanasi	Xac-7	Fruit
8	Western Himalayan Region	Uttarakhand	C.O.A., G.B.Pant University of Agriculture and Technology	Xac-8	Leaf
9	Western Dry land	Rajasthan	Banswara	Xac-9	Fruit
10	Gujarat Plane and Hill Region	Gujrat	Anand Agriculture University, Anand	Xac-10	Leaf
11	Trans Gangentic Plane	Punjab	Ludhiana	Xac-11	Leaf
12	Southern Plateau Hill Region	Karnataka	Belgaon	Xac-12	Leaf
13	Central Plateau and Hill Region	Madhya Pradesh	Jhabua	Xac-13	Twig
14	Eastern Himalayan Region	Meghalaya	Barapani	Xac-14	Leaf
15	Lower Gangentic Plane	West Bengal	Botnical garden Kolkata	Xac-15	Leaf
16	Eastern Coastal Plains and Hills	Odhisa	Agriculture University Bhuvneshwar	Xac-16	Leaf

Pathogenecity

Preparation of bacterial culture

The Sixteen pure bacterial isolates of *Xanthomonas axonopodis* pv.*citri viz.* Xac 1-16 to be tested were inoculated on NA medium. The cultures were incubated cultures at 25^0C for 3 to 5 days prior to inoculation. The 48 hrs old culture was used for the inoculation on NA medium.

Inoculation of bacterial culture

For assessing the varietals reaction against sixteen isolates, Ni ne varieties of acid lime viz.

PDKV Lime, PDKV Bahar, Sai Sarbati, Phule Sarbati, Pramal ini, Chakradhar, Bagalipattu, Tahiti and Kagji lime were selec ted. Inoculation was done by smearing the bacterial culture on leaves at 10 injury points made by pin prick method. The plants were maintained under humid condition. The observations were recorded on the basis of number of pricks made and number of spots exhibited diseased symptoms. Uninoculated injured plants treated with sterilized water surved as control.

Reaction of these isolates on the different citrus cultivars.

Results and discussion

Table 2: Location of diseased samples

S. No.	Location	Code no.	Agro climatic Regions of India
1	Akola	Xac1	Western Plateau Hill Region
2	Nagpur	Xac2	Eastern Plateau Hill Region
3	Pune	Xac3	Western Plateau Hill Region
4	Rahuri	Xac4	West Cost Plane and Ghat Region
5	Dapoli	Xac5	West Coast Plains and Ghat Region
6	Andhra Pradesh	Xac6	Southern Plateau Hill Region
7	Uttar Pradesh	Xac7	Upper Gangentic Plane
8	Uttarakhand	Xac8	Western Himalayan Region
9	Rajasthan	Xac9	Western Dry land
10	Gujrat	Xac10	Gujarat Plane and Hill Region
11	Punjab	Xac11	Trans Gangentic Plane
12	Karnataka	Xac12	Southern Plateau Hill Region
13	Madhya Pradesh	Xac13	Central Plateau and Hill Region
14	Meghalaya	Xac14	Eastern Himalayan Region
15	West Bengal	Xac15	Lower Gangentic Plane
16	Odhisa	Xac16	Eastern Coastal Plains and Hills

Table 3: Reaction of different acid lime cultivars to isolates of *Xanthomonas axonopodis* pv.*citri*

	Days to Initiate water soaked lesions by xac isolates															
Varieties of acid lime	**Xac1**	**Xac2**	**Xac3**	**Xac4**	**Xac5**	**Xac6**	**Xac7**	**Xac8**	**Xac9**	**Xac10**	**Xac11**	**Xac12**	**Xac13**	**Xac14**	**Xac15**	**Xac16**
PDKV Lime	9	7	8	8	5	6	5	7	7	8	8	5	5	4	8	10
PDKV Bahar	8	8	9	5	7	6	8	4	5	8	9	4	8	5	8	10
Sai Sarbati	5	7	6	4	7	8	9	7	6	8	7	6	6	5	9	8
Phule Sarbati	7	8	7	9	8	7	8	7	8	9	7	5	8	4	8	9
Pramalini	8	8	6	5	7	8	6	7	8	8	7	6	8	5	7	8
Chakradhar	3	4	5	6	7	8	9	10	5	6	4	3	9	5	6	4
Bagalipattu	0	0	0	0	0	0	0	0	0	0	0	4	0	4	0	0
Tahiti	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Kagji Lime	9	8	6	7	8	7	5	9	4	7	8	9	8	6	7	9

Sixteen isolates of *Xanthomonas axonopodis* pv. *citri* (Table 3) were assessed for their reactions on acid lime Nine cultivars *viz.* PDKV Lime, PDKV Bahar, Sai Sarbati, Phule Sarbati, Pramalini, Chakradhar, Bagalipattu, Tahiti and Kagji lime by inoculating their pure culture by culture smearing on the leaves. The results (Table 3 and Plate 21) revealed that the

test isolates of variety Chankradhar was most susceptible to all the isolates of Xanthomonas axonopodis pv. citri and expressed symptoms in 3 -10 days after inoculation. Xac1 and Xac12 produced symptoms on cultivar Chankradhar 3 days after inoculation followed by Xac 2, Xac11 and Xac16. However only two Xac isolates viz. Xac12 and Xac 13 were produced symptoms on cultivar Bagalipattu and expressed symptoms within 4 day of inoculation. The isolate Xac12 and Xac14 represented distinct nature of virulence as it initiated water soaked lesion within three to six days. The result revealed that all the isolates were able to induce water soaked lesion on eight acid lime varieties except Tahiti. The isolate Xac12 and Xac14 represented distinct nature of virulence as it initiated water soaked lesion within three to six days.

Tahiti was found resistant against all isolates as none of the isolate was able to cause the disease. Therefore it should consider as resistant cultivar for all the isolates of Xanthomonas axonopodis pv. citri. Rest of the eight acid lime varieties showed susceptible reaction to test isolates. These findings are similar to the results of Das, (2002) [1] who reported pathogenic variability among the isolates of Xanthomonas axonopodis pv. citri and categorized them based on their virulence as highly virulent, moderately virulent and less virulent.

Variable reaction of Xanthomonas axonopodis pv. citri on 30 species and citrus varieties were also recorded by Prasad et al. (1978) [8], which can be used to differentiate the group of isolate into strains.

Katkar *et al*. (2016) studied the pathogenic variability amongst the fifteen isolates Xanthomonas axonopodis pv. citri,by inoculating on four different varieties of acid lime viz. Saisharbati, Phulesharbati, Pramalini and PKM-1 and found that all varieties were found susceptible to all the fifteen isolates of Xanthomonas axonopodis pv.citri.

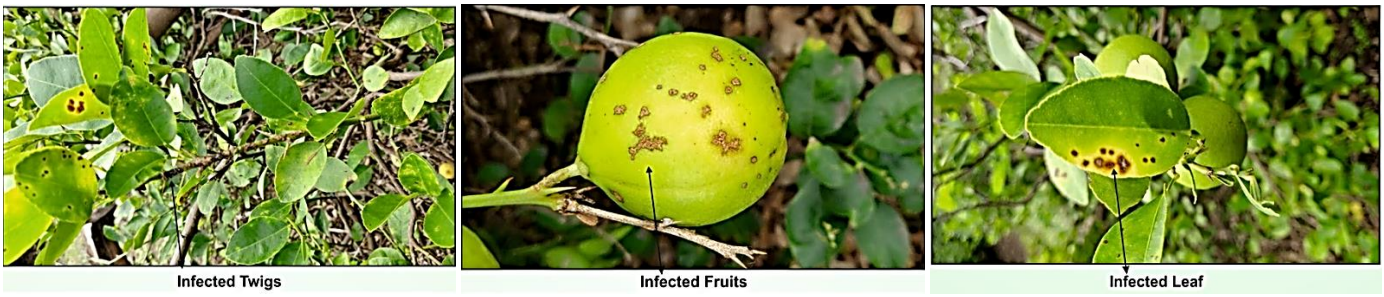

Plate 1: Plant parts used for isolation of pathogen

Plate 2: Pure culture of Xanthomonas axonopodis pv. citri

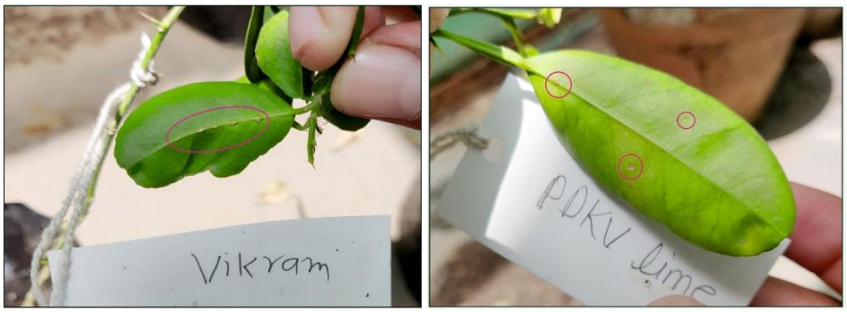

Plate 3: Reaction of selected isolates on the different citrus cultivars

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