

# Antibiotic sensitivity against *Xanthomonas axonopodis* pv. *citri* by using Hi media disc

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## ABSTRACT

Citrus canker disease is of regular occurrence on several citrus cultivars in varying degrees of incidence depending on the climatic conditions. The bacterium, *Xanthomonas* causes different symptoms ranging from pustules to necrotic lesions consisting of erumpent corky tissue surrounded by water soaked tissues and yellow halo on leaves, stems and fruits. Sensitivity of different antibiotics were tested against *Xanthomonas axonopodis* pv. *citri* isolates (*Xac*). using eight different Hi-media disc viz., HX006, HX007, HX010, HX032, HX038, HX060, HX067, HX069. Each Hi-media disc having six different antibiotics of different concentration have been tested against *Xac* isolates. The causal pathogen *Xanthomonas axonopodis* pv. *citri* showed sensitivity to most of the antibiotic tested. Hi-media disc HX060 having antibiotic Imepenem (10mg) and Ticarcillin (70mg) showed maximum zone of inhibition (42mm). However, no effect of antibiotics i.e. Co-Trimoxazole, Nofloxacin, Cefoxitin, Erythromycin observed against *Xac*.

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## INTRODUCTION

Citrus has an important value as fruit crop. Present day citrus is delectable, juicy, and seedless is of great nutritional significance as well (Khan *et al.*, 1992). Additionally, it possesses enormous therapeutic qualities (Chaudhry *et al.*, 1992). Citrus is a member of Rutaceae family and grown in varying densities in countries with tropical or subtropical climates. It is used as best source of vitamin C, sugars, amino acids and other nutrients (Ahmad and Khan, 1999). The most important commercial citrus cultivars in India are the mandarin (*Citrus reticulata* blanco) followed by sweet orange (*Citrus sinensis* osbeck) *aurantifolia* swingle

and acid lime (*Citrus*). Citrus canker is one of the most destructive and predominant disease of acid lime in Vidharbha region of Maharashtra. The disease appears in the form of tiny circular raised necrotic lesions on the leaves and also infects the twigs, petioles, branches, fruits stalks and fruits. A diagnostic yellowish halo often surrounds the lesion on leaves. Severe infection results in defoliation, dieback, and deformation of fruits. *Xanthomonas campestris* pv. *citri* is a rod shaped gram negative bacterium with single polar flagellum. The growth of this bacterium is obligatorily aerobic, while maximum temperature for growth is 35 to 39°C (Mehrotra, 1980 and Whiteside *et al.*, 1988).

## MATERIAL AND METHODS

### Collection, isolation of citrus canker diseased sample :

The suspected diseased samples of acid limes were collected during July –October, 2013. The fresh infected leaves sample were used for isolation employing tissue isolation method. For isolation of *Xanthomonas axonopodis* pv. *citri*. Nutrient agar medium was used.

### Maintenance of bacterial culture :

The respective bacterial cultures were maintained on NA medium at room temperature by adopting subsequent subculturing at periodical, regular intervals. Three days old cultures were used for further studies.

### Identification of the pathogen :

The identification of the pathogen involved in causing of citrus canker in acid lime was determined by conducting studies on its morphological, biochemical, cultural and physiological features of the pathogen as per standard microbiological procedures.

### Antibiotic sensitivity against *Xanthomonas axonopodis* pv. *citri* by Hi-media disc :

Sensitivity of different antibiotics were tested against *Xanthomonas axonopodis* pv. *citri* (Xac). using eight different Hi-media disc viz. HX006, HX007, HX010, HX032, HX038, HX060, HX067, HX069. The bacterium *Xanthomonas axonopodis* pv. *citri* was multiplied by inoculating the loopful culture in 250 ml conical flask containing 100 ml of nutrient broth medium. The inoculated flasks were incubated at 280°C for 72 hours. The 20 ml bacterial suspension was added to molten cooled 1000 ml nutrient agar medium when the temperature was around 38-400°C. The seeded medium was thoroughly mixed and poured into the sterilized Petriplates and plates were allowed to solidify. Different Hi-media disc transferred onto the surface of the seeded medium in Petriplates. The plates were incubated at 25-27°C for 72 hours and observed for the zone of inhibition around the different Hi-media disc.

## RESULTS AND DISCUSSION

Each Hi-media disc having six different antibiotics of different concentration have been tested against Xac isolates. The causal pathogen *Xanthomonas axonopodis* pv. *citri* showed sensitivity to most of the antibiotic tested. Hi-media disc HX060 having antibiotic Imepenem (10 mg) and Ticarcillin (70 mg) showed maximum zone of inhibition (42 mm), however no effect of antibiotics i.e. Co-Trimoxazole, Nofloxacin, Cefoxitin, Erythromycin observed against Xac1(Akola) (Fig. 1). Present findings corroborates with the findings of Isono (1984). Sometimes copper compounds are mixed with

**Table 1 : Sensitivity of different antibiotics against *Xanthomonas axonopodis* pv. *citri***

|       | Antibiotic             | Conc. (mg) | Zone of inhibition in mm |
|-------|------------------------|------------|--------------------------|
| HX007 | NIT (Nitrofurantion)   | 30         | 15.00                    |
|       | NET (Netillin)         | 30         | 30.33                    |
|       | NA (Nalidixic acid)    | 30         | 35.33                    |
|       | CAZ (Cefalozidime)     | 30         | 30.66                    |
|       | CIP (Ciprofloxacin)    | 5          | 36.33                    |
|       | AK (Amikacin)          | 30         | 36.00                    |
| HX010 | CAZ (Cefalozidime)     | 30         | 32.33                    |
|       | CTX (Cefatoxime)       | 30         | 32.33                    |
|       | LE (Levofloxacin)      | 5          | 33.00                    |
|       | AT (Aztreonam)         | 30         | 32.00                    |
|       | AK (Amikacin)          | 30         | 33.00                    |
|       | IPM (Imipenem)         | 10         | 32.33                    |
| HX060 | CTR (Ceftriaxone)      | 30         | 39.00                    |
|       | CPZ (Cefoperazone)     | 75         | 34.66                    |
|       | CX (Cefoxitin)         | 30         | 32.66                    |
|       | IPM (Imipenem)         | 10         | 42.00                    |
|       | TCC (Ticarcillin)      | 75         | 42.00                    |
|       | CPM (Cefepime)         | 30         | 35.00                    |
| HX067 | CF (Cefaclor)          | 30         | 29.33                    |
|       | CTR (Ceftriaxone)      | 30         | 37.66                    |
|       | CXM (Cefuroximeaxetil) | 30         | 22.00                    |
|       | CX (Cefoxitin)         | 30         | -                        |
|       | TCC (Ticarcillin)      | 75         | 40.00                    |
|       | AK (Amikacin)          | 30         | 37.33                    |
| HX069 | C (Chloramphenicol)    | 30         | 11.33                    |
|       | CT (Cefotaxime)        | 30         | 35.00                    |
|       | TE (Tetracycline)      | 30         | 37.33                    |
|       | NX (Norfloxacin)       | 10         |                          |
|       | COT (Co-Trimoxazole)   | 75         | 9.00                     |
|       | AMP (Ampicillin)       | 10         | 12.33                    |
| HX032 | B (Bacitracin)         | 30         | 12.00                    |
|       | C (Chloramphenicol)    | 10         | 11.00                    |
|       | P (Penicillin G)       | 30         | 31.33                    |
|       | PB (Polymyxin B)       | 300        | 32.00                    |
|       | GEN (Grntamycin)       | 10         | 37.00                    |
|       | N (Neomycin)           | 30         | 28.66                    |
| HX038 | AMC (Augmentin)        | 30         | 40.00                    |
|       | E (Erythromycin)       | 10         |                          |
|       | C (Chloramphenicol)    | 30         | 11.00                    |
|       | OF (Ofloxacin)         | 5          | 37.33                    |
|       | COT (Co-Trimoxazole)   | 25         |                          |

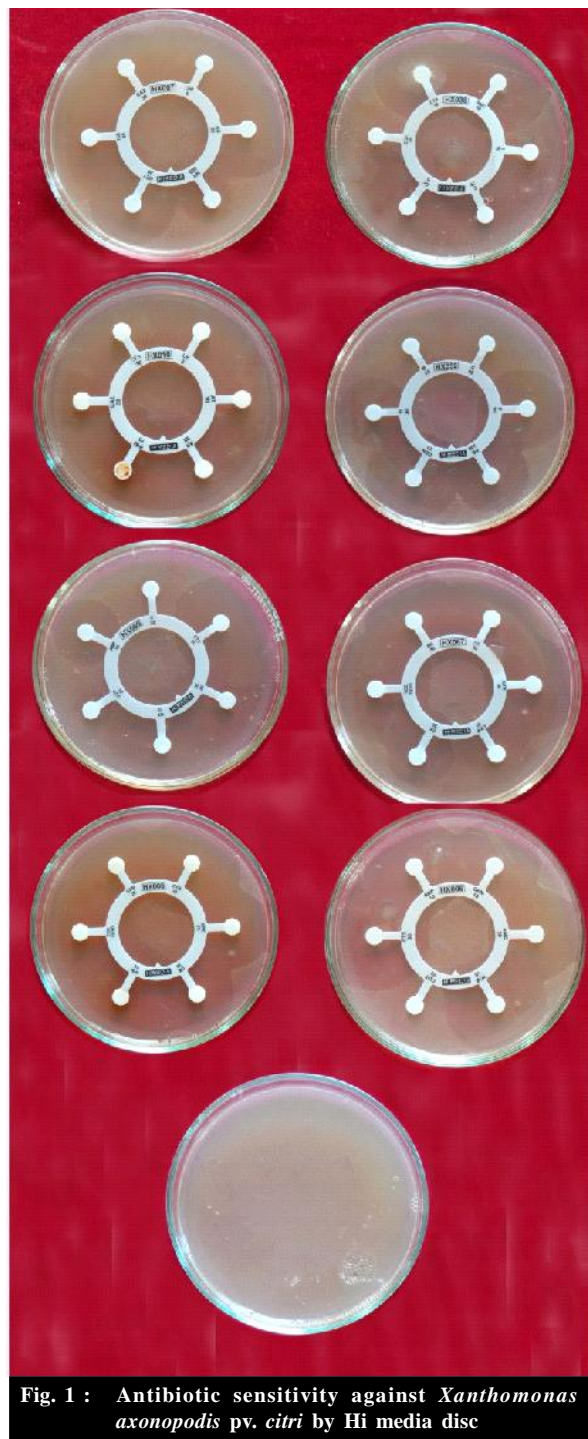


Fig. 1 : Antibiotic sensitivity against *Xanthomonas axonopodis* pv. *citri* by Hi media disc

antibiotics such as streptomycin or oxytetracycline to control bacterial spot. McManus *et al.* (2002). The broad antibacterial activity of dealanylascamycin against various gram-negative and gram-positive bacteria, ascamycin showed selective toxicity against *Xanthomonas citri* and *X. oryzae*. Both ascamycin and dealanylascamycin inhibited the protein

synthesis of *X. citri*, but only dealanylascamycin inhibited that of *Escherichia coli* Fernanda *et al.* (2014). The antibiotic activity of the F3 fraction containing an organometallic compound was tested on Xap *in vitro* and in a greenhouse conditions. Plants were sprayed with F3 before or after Xap infection and the results showed changes in exopolysaccharides and cell morphology. The F3 concentration of 450  $\mu\text{g mL}^{-1}$  was more effective. The results showed that F3 fraction could be a new alternative to control bacterial spot.

#### Conclusion :

The antibiotic sensitivity against *Xac* using Hi-media disc HX060 having antibiotic Imepenem (10mg) and Ticarcillin (70mg) showed maximum zone of inhibition (42mm) however no effect of antibiotics *i.e.* Co-Trimoxazole, Nofloxacin, Cefoxitin, Erythromycin observed against *Xac* isolates.

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