The Pharma Innovation Journal 2023; 12(7): 2915-2920

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(7): 2915-2920

www.thepharmajournal.com
Received: 07-04-2023
Accepted: 19-05-2023

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In vitro management of citrus canker caused by *Xanthomonas axonopodis pv. citri* through antibiotic sensitivity

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Abstract
The goal of the current study was to determine how sensitive to antibiotics *Xanthomonas axonopodis pv. citri* was. The paper disc method was used *in vitro* to examine antibiotic sensitivity. Different antibiotics and substances, including streptomycin sulphate, Streptocycline, Kasugamycin, and copper oxychloride, were used in that. Combination of copper oxychloride (0.3%) + Streptocycline (200 ppm) and copper oxychloride (0.2%) + streptomycin sulphate (200 ppm) significantly outperformed the other two combinations in terms of preventing bacterial growth.

Keywords: *Antibiotics, Xanthomonas,* Growth inhibition, Kasugamycin, bacterial

Introduction
The world's most important fruit crop is citrus. Today's citrus is delicious, juicy, and seedless, and it also has significant nutritional value (Khan *et al*., 1992) [18]. It also has tremendous therapeutic benefits (Chaudhary *et al*, 1992) [9]. It serves as the best source of nutrients, including sugars, amino acids, and vitamin C. (Ahmad and Khan, 1999) [2]. The mandarin is the most significant commercial citrus cultivar in India, followed by the sweet orange and acid lime. In India Commercially acid lime is grown in Khara district of Gujarat, Akola and Amravati districts of Maharashtra and Periyakulam in Tamil Nadu. Even though citrus crops are held in high regard, a number of issues threaten their current status. One of them is low production brought on by biotic and abiotic stresses. Numerous diseases, including citrus canker, gummosis, citrus decline, citrus tristeza virus, greening, etc., attack citrus plants. The most significant threat to citrus plants is *Xanthomonas axonopodis* pv.*citri*, which causes citrus canker (Sahi *et al*., 2007) [19].
Depending on the climatic conditions, the citrus canker disease affects several citrus cultivars on a regular basis in varying degrees of incidence. The bacterium Xanthomonas causes a variety of symptoms on leaves, stems, and fruits, from pustules to necrotic lesions with erupted corky tissue encircled by water-soaked tissues and a yellow halo (Zekri *et al*., 2005) [20]. Due to the severity of the disease on susceptible varieties, less fruit is produced and has a lower market value due to defoliation, premature fruit drop, and blemished fruit (Zekri *et al*., 2005) [20]. There are numerous pathovars and variants of the bacterium *Xanthomonas axonopodis* that cause citrus canker disease (Graham *et al*., 2004) [17]. The species of bacteria in the genus Xanthomonas are gram-negative, straight rod-shaped, motile with a single polar flagellum, and obligately aerobic, with a maximum growth temperature tolerance of 35 to 39 °C (Whiteside *et al*., 1988) [21]. Serious plant pathogens are Xanthomonas species members. Citrus canker is a worldwide disease, so different aspects of it have been potentially addressed and adequately researched in different parts of the world. As a result, it has become necessary to replace the traditional method of disease management (chemical control) with safer and more environmentally friendly management strategies (biological and genetic control). As a result, there would be less of a reliance on harmful to the environment chemicals. In the current study, various antifungal and antibacterial antibiotics, including streptomycin sulphate, Streptocycline, copper oxychloride, and Kasugamycin, were used to treat the disease *in vitro*.

Material and Methods
Collection and isolation of diseased plant samples Collection of diseased samples
Eight diseased acid lime samples infected with citrus canker were collected from various locations throughout Maharashtra state between July and October.

Namely, Nagpur, Bhandara, Amravati, Akola, Washim, Pune, Rahuri, and Dapoli, and isolated the bacterium on nutrient agar medium.

Identification of the pathogen

In accordance with accepted microbiological practises, the pathogen's morphological, cultural, and physiological characteristics were used to identify *Xanthomonas axonopodis* pv. *citri.*

Preparation of bacterial culture

On NA medium, the sixteen *Xanthomonas axonopodis pv. citri* pure bacterial isolates (Xac 1- 16) that would be tested were inoculated. Prior to inoculation, the cultures were incubated for 3 to 5 days at 250C. For the inoculation on NA medium, the 48-hour-old culture was utilized.

In vitro **efficacy of different antibiotics and fungicides against *Xanthomonas axonopodis pv. citri* by paper disc method**

The eight isolates (Xac) were tested for sensitivity using a modified paper disc assay. Streptomycin sulphate, Streptocycline, copper oxychloride, and Kasugamycine were the antibiotics and fungicides that were extracted and freshly prepared in sterile distilled water. A 150 ml conical flask containing 50 ml of nutrient broth medium was inoculated with a Loopful culture of the bacterium *Xanthomonas axonopodis pv. citri* to multiply it. For 72 hours, the inoculated flasks were incubated at 27 20 °C. When the NA media had cooled and was just about to solidify, 10 ml of the prepared bacterial suspension from each isolate was added. After thoroughly shaking, the medium containing the bacterial suspension was immediately poured into sterile Petri plates and allowed to set. The fungicide and antibiotic concentrations were made. Before use, the filter paper disc (Whatman No. 42), which has a diameter of 5 mm, was prepared and Sterilised. The Sterilised filter paper discs were placed on the surface of the seeded medium in Petriplates after five minutes of soaking in the corresponding chemical concentrations. The plates were incubated for 72 hours at 27 °C, and the development of an inhibition zone around the filter paper discs was monitored. The control group consisted of the paper discs soaked in sterile distilled water. The resulting results were statistically analysed.

Table 1: Treatement details.

Sr. No	Treatment Details	Concentration
1.	Streptocycline	100 ppm, 200 ppm
2.	Streptomycin sulphate	100 ppm, 200 ppm
3.	Kasugamycin	100 ppm, 200 ppm
4.	Copper oxychloride	0.2%, 0.3%
5.	Copper oxychloride +Streptocycline.	0.2%+ 100 ppm, 0.2%+ 200 ppm
6.	Copper oxychloride + Streptomycine sulphate	0.3%+100 ppm 0.3%+200 ppm

Results and Discussion

Efficacy of different antibiotic and chemicals against *Xanthomonas axonopodis* pv. *citri*

Antibiotics and other substances were tested using the Paper disc method against eight isolates of *Xanthomonas axonopodis pv. citri* (Xac 1 to Xac 8).

The findings showed that treatments T10 (copper Oxychoride (0.2%) + Streptocycline (200 ppm)) and T12 (copper oxychloride (0.3%)+Streptomycine sulphate (200 ppm)) were both found to be significantly effective in preventing the growth of the test pathogen. The treatment number T12 (Copper oxychloride (0.3%) +Streptomycine sulphate (200 ppm)] for the isolate Xac7, followed by T10 (Copper Oxychloride (0.2%) + Streptocycline (200 ppm)] for the isolate Xac1, produced the largest zone of inhibition (32 mm) (26.66 mm).

However, it was discovered that using the fungicide COC and the antibiotic Kasugamycine alone at different concentrations was insufficient to inhibit the zone. The data in Table 2 also show that Treatment No. T9, which is COC + Streptocycline (Hindustan Antibiotics Ltd.) alone, was found to inhibit the zone of inhibition 90:10 (Streptomycine sulphate I.P and tetracycline hydrochloride, respectively) (0.2%+100 ppm) and T11 (0.3%+100 ppm) after T10 and T12.

The lowest zone of inhibition i.e zero percent were recorded in T_4 in isolate Xac4, T_5 in isolate Xac1 and Xac4, T_6 in isolate Xac1 and Xac4.

The current results are consistent with those of Sharma *et al.* (1981), who found that, when tested *in vitro*, the combination of streptocycline and copper oxychloride was most effective in preventing the growth of *Xanthomonas vesicatoria*. A similar finding was also made by Manjula *et al* (2002) When bactericides were tested *in vitro* for effectiveness against Xanthomonas axonopodis pv. Punicae, Copper oxychloride @ 2000 ppm was found to be only moderately effective. According to Das (2003), COC (0.3%) plus Streptomycine (100 ppm) stopped *Xanthomonas axonopodis pv. citri* from growing.

Abhang *et al.* (2018) [1] stated that the Copper oxychloride (0.2%) + streptomycin sulphate (200 ppm) was found significantly effective in inhibiting the growth of citrus canker causing bacteria *Xanthomonas axonopodis* pv. *citri.* Antre *et al.* (2016) [7] investigated that Copper oxychloride (0.3%) + Streptomycin sulphate (500 ppm) was found significantly superior in inhibiting the growth of *Xanthomonas axonopodis* pv. *punicae* with 14.33 mm zone of inhibition.

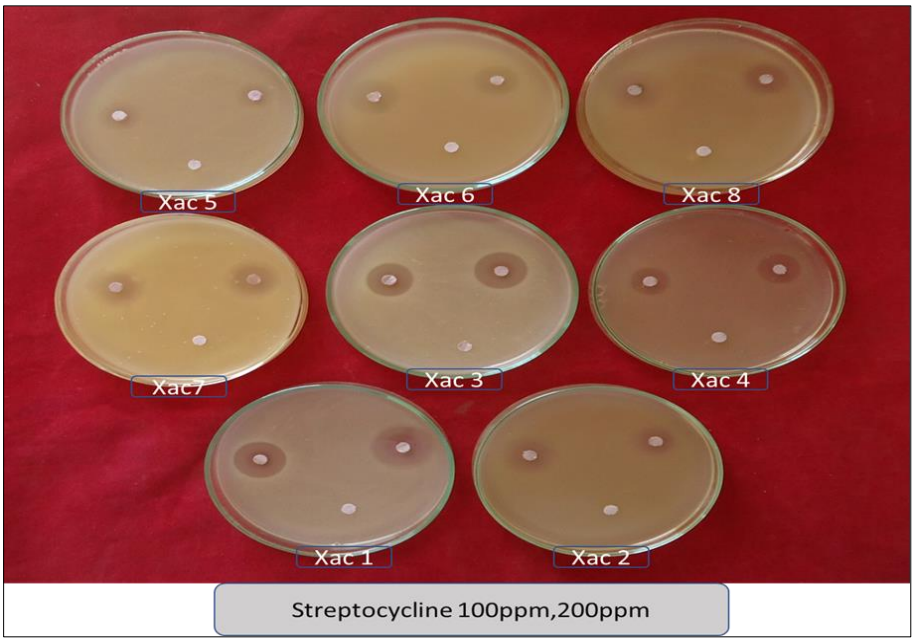

Fig 1: Streptocycline 100ppm, 200pm

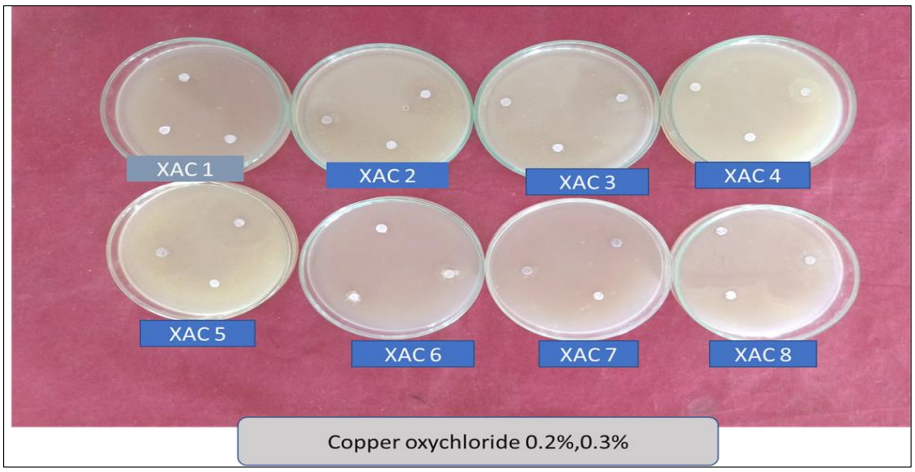

Fig 2: Copper oxychloride 0.2%, 0.3%

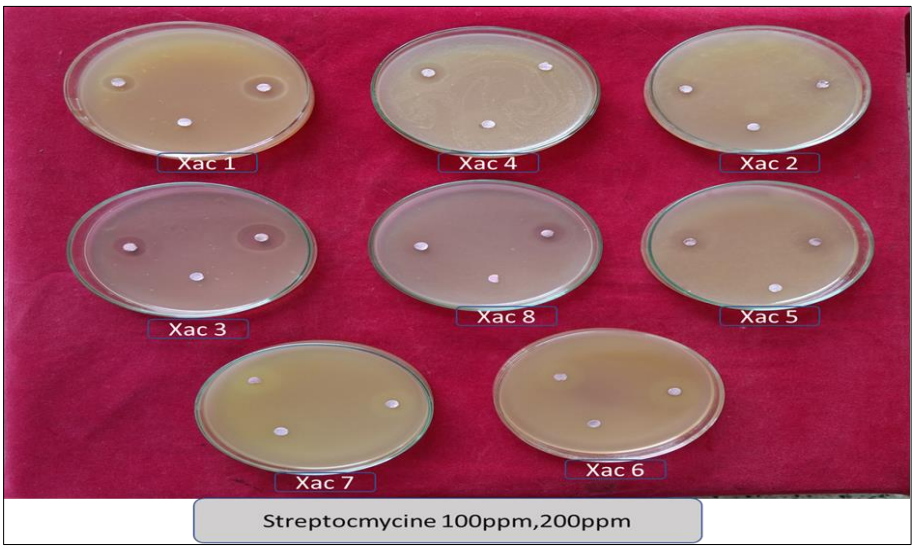

Fig 3: Streptomycine 100ppm, 200pm

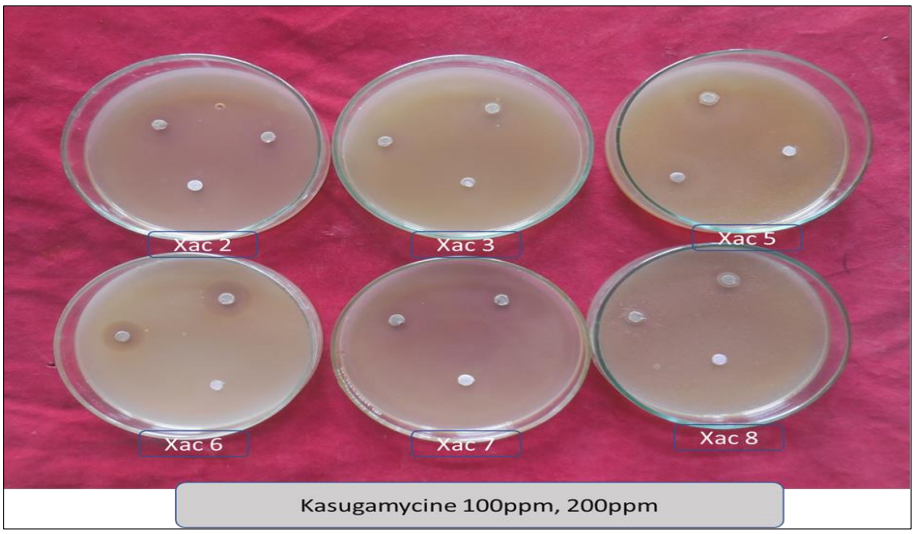

Fig 4: Kasugamycine 100ppm, 200pm

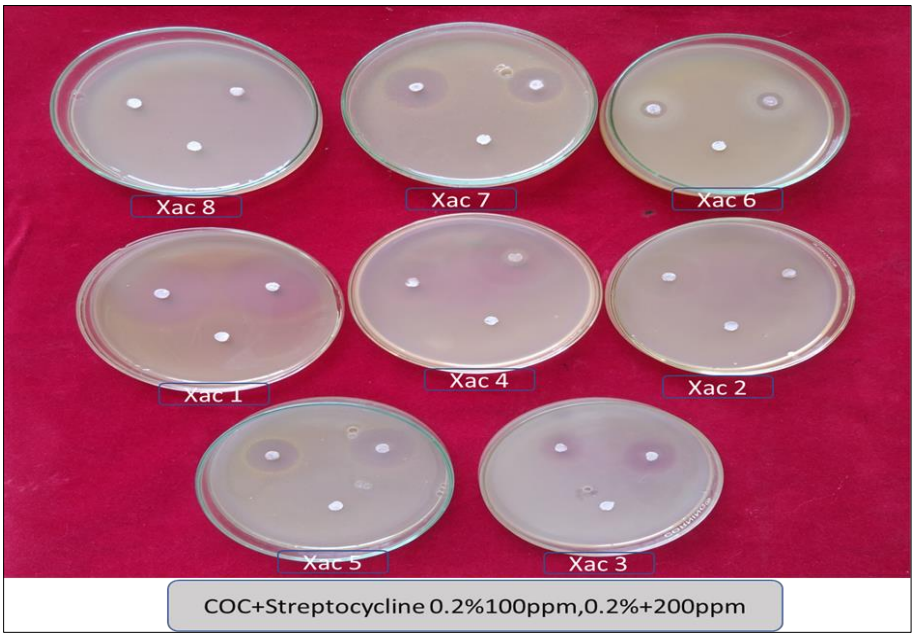

Fig 5: COC+Streptocycline 0.2% 100ppm, 0.2%+200ppm

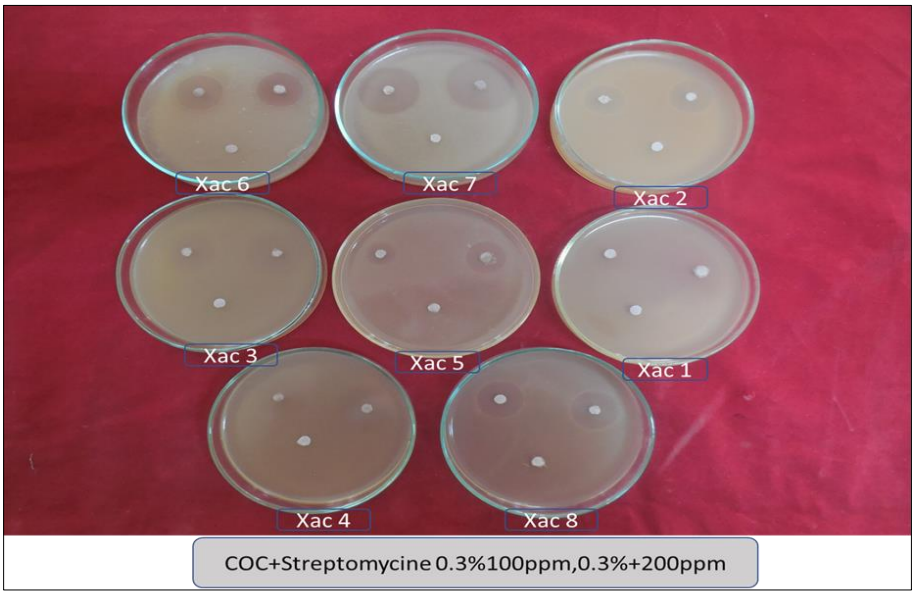

Fig 6: COC+Streptocycline 0.3% 100ppm, 0.3%+200ppm

Table 2: Efficacy of different antibiotics and chemicals against eight isolates of *Xanthomonas axonopodis* pv. *citri*.

Treatment no.	Treatments with conc.	Zone of inhibition (mm) × average of three replication							
		Xac1	Xac2	Xac3	Xac4	Xac5	Xac6	Xac7	Xac8
T_1	Streptocycline100 ppm	17.00	16.00	17.00	13.00	15.00	17.33	13.00	18.66
T_2	Streptocycline 200ppm	18.33	16.66	19.00	18.66	18.00	19.00	18.66	21.00
T_3	Streptomycine sulphate 100ppm	16.00	10.00	8.66	15.00	10.00	12.66	11.00	10.66
T_4	Streptomycine sulphate 200ppm	20.00	13.00	18.66	0.00	13.66	18.00	17.66	16.66
T_5	Kasugamycine 100ppm	0.00	14.00	5.66	0.00	4.00	16.00	10.33	5.00
T_6	Kasugamycine 200ppm	0.00	18.00	9.00	0.00	6.00	19.00	13.66	6.66
T_7	Copper oxychloride 0.2%	4.33	12.00	0.00	2.33	9.00	6.66	6.00	5.00
T_8	Copper oxychloride 0.3%	77.00	15.00	6.00	8.00	12.00	9.00	8.00	11.00
T_9	COC+Streptocycline 0.2%+100ppm	26.00	18.33	13.33	12.33	21.66	22.00	17.00	13.00
T_{10}	COC+Streptocycline 0.2%+200ppm	26.66	21.66	17.66	20.33	25.00	24.00	21.33	16.00
T_{11}	COC+Streptocycline sulphate 0.3%+100ppm	13.00	21.33	17.00	18.00	12.66	19.00	28.00	21.66
T_{12}	COC+Streptocycline sulphate 0.3%+200ppm	19.00	22.00	20.00	22.66	18.33	21.66	32.00	25.00
T_{13}	Control	0	0	0.00	0.00	0.00	0.00	0.00	0.00
	'F' test	SIG	SIG	SIG	SIG	SIG	SIG	SIG	SIG
	SE (m) +	0.03	0.02	0.03	0.04	0.02	0.03	0.03	0.03
	CD (P) = 0.01	0.13	0.08	0.13	0.14	0.09	0.11	0.10	0.12

*Values in parenthesis are square root transformed

Conclusion

By measuring the growth using the paper disc method, the effectiveness of various antibiotic and chemical combinations against *Xanthomonas axonopodis pv. citri* was evaluated *in vitro*. The results showed that treatments no. T10 (copper Oxychoride [0.2%]+ streptocycline [200 ppm]) and T12 [copper oxychlor [0.3%]+ Streptomycine [200 ppm]] were found to be significantly effective in preventing the growth of the test pathogen. The treatment number T12 (Copper oxychloride (0.3%)+Streptomycine sulphate (200 ppm)] for the isolate Xac7, followed by T10 (Copper oxychloride (0.2%)+ streptocycline (200 ppm)] for the isolate Xac1, produced the largest zone of inhibition (32 mm) (26.66 mm). Additionally, it was discovered that treatments T9, which consisted of COC + Streptocycline alone, and T11, which consisted of COC + Streptomycine sulphate (0.3%+100 ppm), inhibited the zone of inhibition following treatments T10 and T12.

Future scope

Efficacy of different combination of antibiotics and chemicals against *Xanthomonas axonopodis* pv. *citri* was assessed *in vitro*. We can also use superior antibiotics and chemicals in inhibiting growth of bacteria.

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