

Efficacy of antibiotics and bactericides against *Xanthomonas axonopodis* pv. *citri* causing bacterial canker disease of acid lime (*Citrus aurantifolia*)

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Summary

Diseases are the major constraints for citrus production which impede the fruit yield and quality. Citrus suffers from a number of diseases caused by fungi, bacteria, viruses and virioids. Among these diseases, canker caused by *Xanthomonas axonopodis* pv. *citri*, foot rot or gummosis caused by *Phytophthora* spp., twig blight caused by *Diplodia natalensis* and *Fusarium* spp. Three antibiotics and three bactericides viz., Streptocycline, Kasugamycin 5% + Copper oxychloride 45% WP, Kasugamycin, Bacterimycin, Copper oxychloride 50% WP, Copper hydroxide 53.8% W/W were evaluated for their effectiveness in controlling the growth of *Xanthomonas axonopodis* pv. *citri* at the Department of Plant Pathology, College of Agriculture, Ummendganj-Kota, (Agricultural University, Kota) Rajasthan. Inhibition was highest in case of copper oxychloride 50% WP (34.20 mm) followed by bronopol 27% (28.90 mm) and streptocycline 90% W/W (12.48 mm) at 500 ppm concentration and no inhibition zone was found in kasugamycin. All the antibiotics/bactericides inhibited bacterial growth at higher concentrations.

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Keywords: Citrus (*Citrus aurantifolia*), *Xanthomonas axonopodis* pv. *citri*, antibiotics/bactericides.

Introduction

The word 'Citrus' probably originated from the Greek word 'Kedros', which originally meant 'Cedar' or 'Cedarcone' Citrus fruits belonging to the genus of the family Rutaceae and sub-family Aurantiodeae. Lime is rich in vitamins, minerals and alkaline salts, but not so much in fruit sugars, which play a role of health-promoting ingredients in the human diet. Excellent sharbat is prepared from juice. In India, acid lime is one of the most important citrus fruits grown. It constitutes nearly 20% of total citrus production in India (Ghosh et al. 2010).

In India, area under citrus fruit cultivation is 1.08 million hectares (MH), with production of 14.26 million tonnes. Citrus fruits include important crops such as oranges, lemons, grapefruits, pomelos and limes. In Rajasthan, area under acid lime cultivation is 3030 ha, with a production of 21749 tonnes (Anon. 2023). Citrus canker is an important disease affecting citrus species. In India, citrus canker was first reported from Punjab

(Luthra and Sattar, 1942) and is now known to be prevalent in Tamil Nadu, Andhra Pradesh, Karnataka, Rajasthan, Assam, Madhya Pradesh, Uttar Pradesh and Himachal Pradesh (Naga Lakshmi et al. 2014). The bacterium (*Xanthomonas axonopodis* pv. *citri*) is rod-shaped, gram-negative with a single polar flagellum. It is obligately aerobic, non-spore former and produces yellow colonies on nutrient agar medium and it can withstand higher temperature of 35 to 39°C (Ramakrishnan 1954; Isokar et al. 2020; Sharma et al. 2018).

Materials and Methods

Isolation, Purification and Identification of Pathogen

The *Xanthomonas axonopodis* pv. *citri* (vauterin et al. 1995) was isolated by tissue isolation method. The diseased samples were washed thoroughly with tap water and allowed to dry under shade. The infected leaves part along with healthy tissues was cut into small pieces and was surface sterilized with 0.1 per cent mercuric chloride (HgCl₂) solution for one minute and washed three times serially with sterile distilled water to remove the traces of mercuric chloride. The inoculated plates were incubated at 28±2 °C for 72 hrs. After the incubation period, observations were made for the development of well-separated, typical, light yellow-coloured bacterial colonies resembling *Xanthomonas* sp.

Purification and maintenance of bacterial culture

The inoculated plates were incubated at 30°C for 72 hours. Observations were made for the development of well separated typical, bright yellow, mucoid colonies, such pure colonies were further streaked onto the agar slants containing the nutrient agar medium and incubated at 28±2 °C for 72 hrs, later, cultures were

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stored in the refrigerator at 5°C, which served as a stock culture for further studies.

Identification of the pathogen

The identification of the pathogen involved in causing of acid lime canker was determined by conducting studies on its morphological and cultural characters of the pathogen as per standard microbiological procedures such as observation of the bacterial cells under compound microscope.

Observation recorded

Table 1. Treatments details

S. No.	Antibiotics/Bactericides	Concentration (ppm)		
1.	Streptocycline 90% W/W	100	250	500
2.	Conika (Kasugamycin 5% + Copper oxychloride 45% WP)	100	250	500
3.	Kasugamycin (Biomycin)	100	250	500
4.	Bacterimycin	100	250	500
5.	Copper oxychloride 50% WP	100	250	500
6.	Copper hydroxide 53.8% WP	100	250	500
7.	Control	-	-	-

Experimental details

Design: CRD, Treatments: 7 (including control):

Replications: 3.

Preparation of paper disks with formulations

A total of 63 petri plates were filled with this thickly suspended nutrient agar medium containing bacteria. The medium suspension in plates was allowed to solidify. Filter paper discs measuring 6 mm in diameter were soaked separately in the antibiotics/bactericide formulations and then placed onto the surface of the seeded nutrient agar medium. Three discs which were previously dipped in the respective formulation solution in the respective 50 ml flask, were kept equidistant in each petri plate. They were considered as three replications.

Inhibition zone observation

The inoculated plates were first incubated at 5° C in a refrigerator to allow the diffusion of suspension into the medium and then incubated at 28±2°C for 72 hours.

Results and Discussion

The filter paper disc impregnated with desired concentrations viz., 500 ppm, 250 ppm, and 100 ppm of different antibiotics/bactericides discs were placed on the growth of *Xanthomonas axonopodis* pv. *citri*. Observations on the formation of zone of inhibition were recorded at 24 h intervals. The result presented in Table 2 revealed that all the antibiotics/bactericides inhibited bacterial growth at higher concentrations. The inhibition zone range varied from 2 mm to 38 mm. Copper oxychloride 50% WP (16.97) and Bacterimycin (14.23) showed the highest inhibition at 100 ppm and least inhibition showed by Streptocycline 90% W/W, Kasugamycin 5% + Copper oxychloride 45% WP, Kasugamycin, Copper hydroxide 45% WP (0.00). At 250 ppm, Copper oxychloride 50% WP (24.20) followed by

In paper disk method, agar plate was inoculated with test organism i.e., *Xanthomonas axonopodis* pv. *citri*. Then the filter paper disk measuring 6 mm was impregnated with antibiotics and bactericide at a desired concentration and then were placed on the agar surface. Sterile and double distilled water was used as control. The plates were incubated at 28±2°C overnight and zone of inhibition around the disks was measured after 48 hours.

In-vitro evaluation of antibiotics and bactericides against *Xanthomonas axonopodis* pv. *Citri*

Bacterimycin (21.67) and Streptocycline (16.23). least inhibition by Kasugamycin 5% + Copper oxychloride 45% WP, Kasugamycin and Copper hydroxide 53.8% W/W (0.00). At 500 ppm Copper oxychloride 50% WP (34.20), Bacterimycin (28.90), Streptocycline 90%W/W (21.20), Kasugamycin 5% + Copper oxychloride 45% WP (14.67), Copper hydroxide 53.8% W/W (8.97) and least inhibition show by Kasugamycin (0.00).

The mean inhibition was highest in case of copper oxychloride 50% WP at 500 ppm followed by Bacterimycin and Streptocycline. The inhibition showed that the bacterial isolate was highly sensitive towards copper oxychloride 50% WP and Bacterimycin sensitive to Kasugamycin 5% + Copper oxychloride 45% and Copper hydroxide 53.8% W/W moderate sensitive to Streptocycline 90% W/W and resistance to Kasugamycin.

These results conform with the findings of those reported earlier by several workers. Giri et al. (2008) evaluated copper oxychloride (0.2% and 0.3%) + Streptocycline (100 ppm and 200 ppm) against *Xanthomonas axonopodis* pv. *citri*. Among that the minimum disease intensity (12.29%) with maximum disease control. Samavi et al. (2009) reported the bordeaux mixture + mancozeb, copper oxychloride + mancozeb were found effective against citrus (37.63%) was recorded with three sprays of copper oxychloride + streptocycline (0.3% + 100ppm) (Nain et al. 2022). Jadhav and Sharma (2011) carried out studies during 2005-06 and revealed that sprays with Streptocycline (500 ppm) + COC (2000 ppm) were very effective in reducing the mean disease incidence (25.5%) of *Xanthomonas axonopodis* pv. *punicae* followed by Bromopal (500 ppm) + copper oxychloride - 2000 ppm) (33.3%), when compared with control (78.5%).

Table 2. In-vitro efficacy of antibiotics/bactericides against *Xanthomonas axonopodis* pv. *citri* after 24 h of incubation at 28±2°C

S. No.	Antibiotics/Bactericides	Inhibition zone (mm)			Mean	Response
		100 ppm	250 ppm	500 ppm		
1.	Streptocycline 90% W/W	0.00*	16.23	21.20	12.48	Moderate sensitive

		(0.00)**	(23.75)	(27.41)	(17.05)	
2.	(Kasugamycin 5% + Copper oxychloride 45% WP)	0.00 (0.00)	0.00 (0.00)	14.67 (22.50)	4.89 (7.50)	Sensitive
3.	Kasugamycin (Biomycin)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	Resistance
4.	Bacterimycin	14.23 (22.15)	21.67 (27.74)	28.90 (32.51)	21.60 (27.47)	Highly sensitive
5.	Copper oxychloride 50% WP	16.97 (24.32)	24.20 (29.46)	34.20 (35.79)	25.12 (29.86)	Highly sensitive
6.	Copper hydroxide 53.8% W/W	0.00 (0.00)	0.00 (0.00)	8.97 (17.41)	2.99 (5.80)	Sensitive
7.	Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	-
	Concentration means	4.46 (6.64)	8.87 (11.56)	15.42 (19.37)	9.58 (12.53)	
		S. Em ± CD at 0.05%	T 0.94 2.68	C 0.77 2.18	TXC 0.44 1.26	

*Average of three replications, **Figures in parentheses square root transformed values.

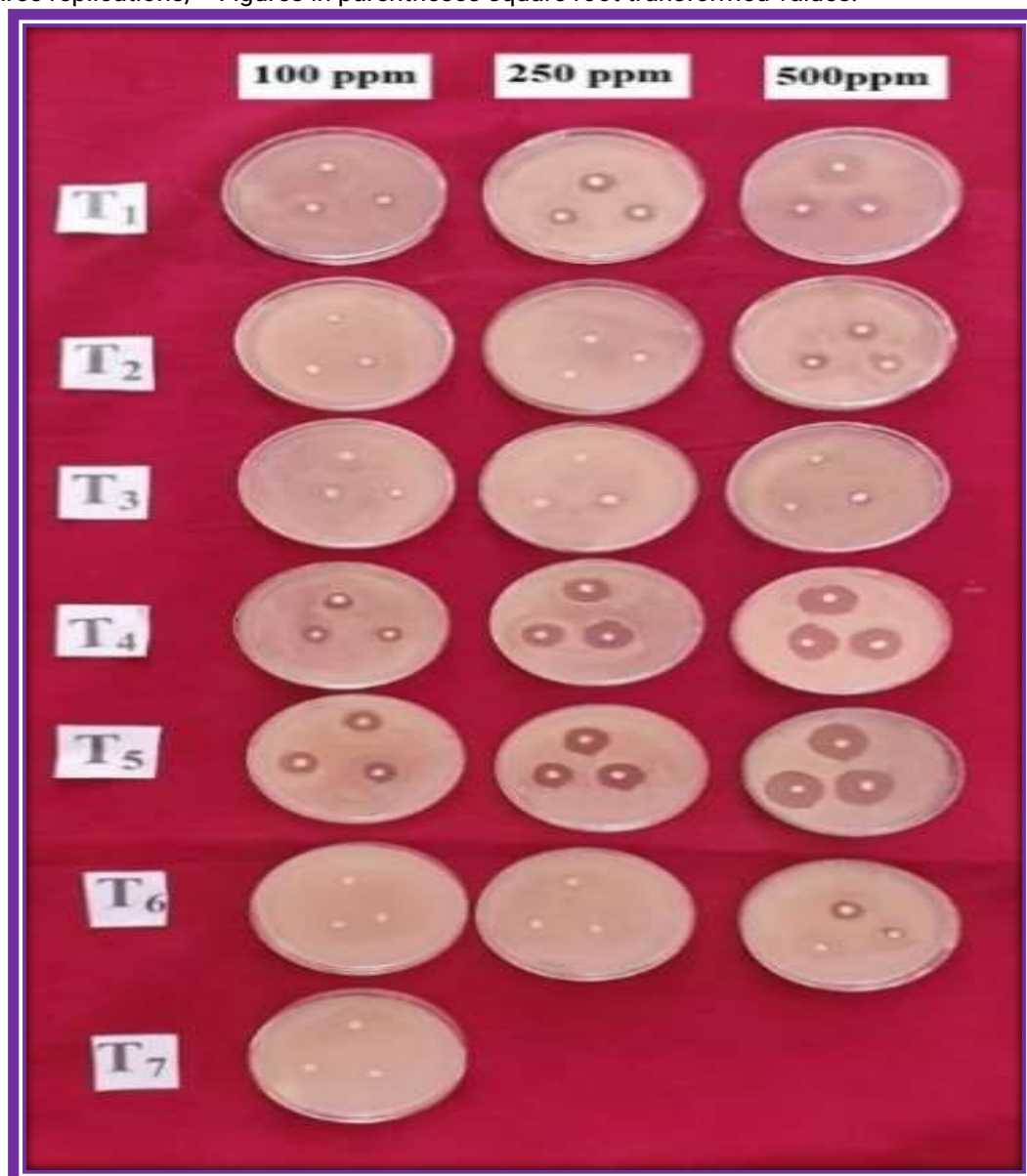


Plate 1. In-vitro efficacy of antibiotics/bactericides against *Xanthomonas axonopodis* pv. *citri*, Here, T₁= Streptocycline 90% W/W, T₂= Conika (Kasugamycin 5% + Copper oxychloride 45% P), T₃= Kasugamycin (Biomycin), T₄= Bacterimycin, T₅= Copper oxychloride 50% WP, T₆= Copper hydroxide 53.8% W/W, T₇= Control

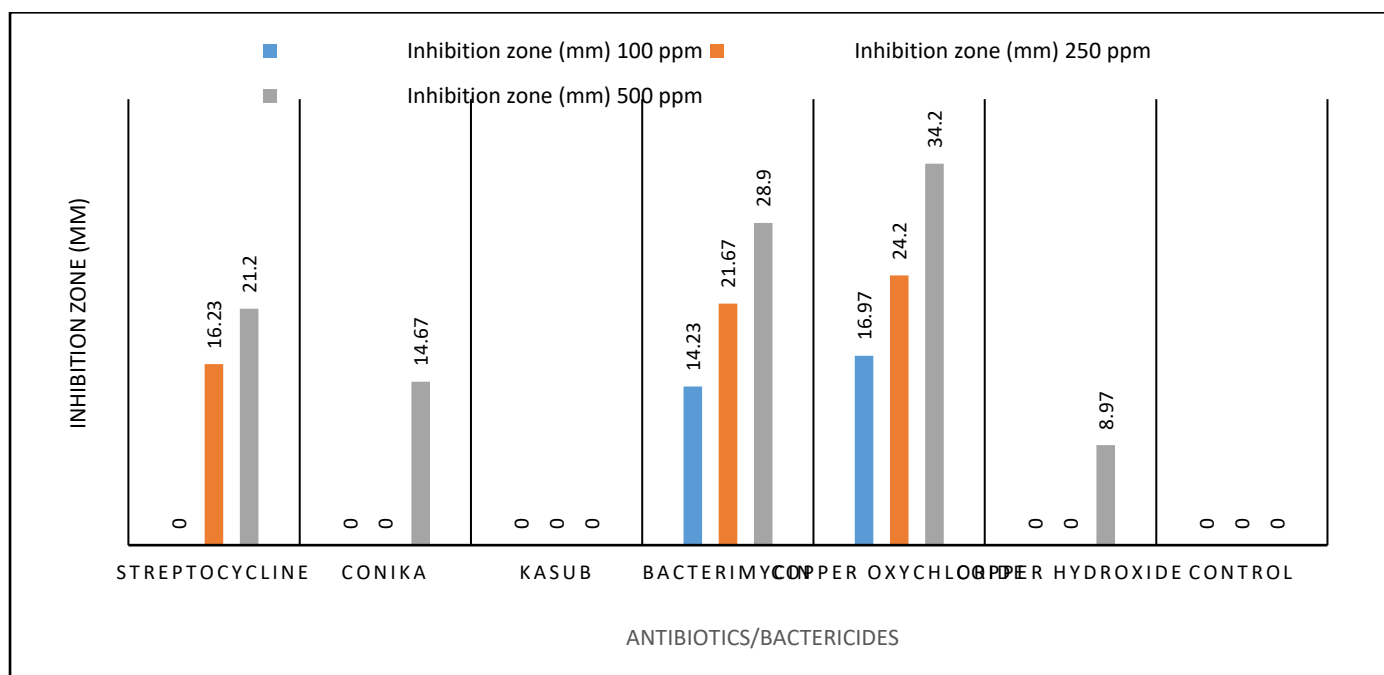


Figure 1. In-vitro efficacy of antibiotics / bactericides against *Xanthomonas axonopodis* pv. *citri* after 24 h of incubation at 28±2°C

Conclusion

From the results obtained on various aspects during the present investigation on bacterial canker disease of acid lime (*Xanthomonas axonopodis* pv. *citri*), following conclusions are being drawn: In all bactericides/antibiotics evaluated, copper oxychloride 50% WP followed by bronopol 27% found effective against *Xanthomonas axonopodis* pv. *citri*.

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