Int.J.Curr.Microbiol.App.Sci (2018) Special Issue-6: 1693-1699

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Special Issue-6 pp. 1693-1699**
Journal homepage: http://www.ijcmas.com

Original Research Article

In Vitro Bioefficacy of Different Antibiotics, Fungicides and Botanicals against (*Xanthomonas axonopodis* pv. *citri*.) Causing Bacterial Canker of Kagzi Lime

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Keywords

Kagzi lime, Bacterial canker, *Xanthomonas citri* pv. *citri*, Antibiotics, Fungicides

A B S T R A C T

Four antibiotics, five antibacterial chemicals and nine botanicals were evaluated by *in vitro* against *Xanthomonas axonopodis* pv. *citri* (Hasse). Among the different antibiotics and antibacterial chemicals, Streptocycline (27.35 mm) and copper oxychloride (25.53 mm) showed maximum average inhibition zone followed by plntomycin (23.21 mm). Among the botaniacals, Ginger *(Z. officinale)* was effective with the Average inhibition zone of (20.04 mm) followed by Neem (19.87 mm). The fungicide carbendazim were found very less effective against the pathogen.

Introduction

Citrus bacterial canker (CBC), caused by *Xanthomonas citri* sub sp. *citri* is one of the most devastating diseases throughout the world that affects many kind of commercial citrus varieties.

The origin of CBC is known but thought to have originated from south-east Asia or India and then widely distributed around the world (Civerolo 1984; Verniere *et al.,* 1998) According to Fawcett and Jenkins (1993), All cultivars of citrus are susceptible to canker, but grape fruit, Mexican lime and lemon are highly susceptible, whereas sour orange and sweet orange are moderately susceptible. Mandarins are moderately resistant (Gottwald *et al.,* 1993).

Material and Methods

Isolation and Identification of the pathogen

Diseased samples showing typical symptoms of bacterial canker on kagzi lime were collected from farmer's field conformed by ooze test and subjected to isolation by streak plate method.

The identification of the *Xanthomonas axonopodis* pv. *citri* was done by morphological, cultural and biochemical features of the pathogen.

The cultures were maintained on Nutrient agar slants and were stored at 5±1°C, their revival were effected at certain intervals as required.

Antibiotics and fungicides sensitivity against *Xanthomonas axonopodis* pv. *citri* by inhibition zone technique

Four antibiotics *i.e.* (each @ 100, 250 and 500 ppm), five fungicides *i.e.* (each @ 1000, 2000 and 3000 ppm) and aqueous phytoextracts (leaf/rhizome /bulb) of nine botanicals (each @ 10 and 20 %) were evaluated *in vitro* by applying inhibition zone assay method and using Nutrient Agar (NA) as basal medium. Nutrient agar medium poured into sterilized glass Petri plates allowed to solidify. One ml of mass multiplied broth culture of test bacterium (2 x 10^8 cfu/ml) was spread with plastic spreaders over the solid surface of NA poured in plates. The solution of the desired concentration of the test antibiotics, fungicides and botanicals was prepared separately. The filter paper discs (Whatman No.42) of 5mm in diameter was soaked separately in the respective chemical solutions for 5-10 minutes and transformed in centre onto the solidified bacterium spread on NA medium in Petri plates. The inoculated plates were kept in the refrigerator at 4^0 C for 4 hours to allow diffusion of the chemical into medium. The untreated control plate containing with the test bacterium spread on NA and inoculated with filter paper disc soaked in distilled water was also maintained then the plates were incubated at 28±1^{0}C for 72 hours and observed for the production of inhibition zone around filter paper discs. The paper disc soaked in sterile distilled water served as control. The results obtained were analyzed statistically. (Raju *et al.*, 2012)

Results and Discussion

Efficacy of antibiotics

Results (Table 1) indicated that the antibiotics tested at various concentrations (each @ 100, 250 and 500 ppm) significantly inhibited growth of *X. axonopodis* pv. *citri*, over untreated control.

At 100 ppm, bacterial inhibition zone was ranged from 9.94 mm (Kasugamycin) to 17.36 mm (streptocycline). However it was significantly highest with streptocycline (17.36 mm), followed by Plantomycin (12.81 mm) and 2-Bromo-2 Nitro propane-1,3-diol (10.19 mm); whereas, significantly least inhibition zone was found with Kasugamycin (9.94 mm).

At 250 ppm, bacterial inhibition zone was ranged from 11 mm (Kasugamycin) to 20.28 mm (streptocycline).

However it was significantly highest with streptocycline (20.28 mm), followed by 2-Bromo-2 Nitro propane-1,3-diol (15.92 mm) and Plantomycin (15.53 mm); whereas, significantly least inhibition zone was found with Kasugamycin (11.00mm).

At 500 ppm, bacterial inhibition zone was ranged from 13.17 mm (Kasugamycin) to 20.08 mm (streptocycline).

However it was significantly highest with streptocycline (20.08 mm), followed by Plantomycin (18.50 mm) and 2-Bromo-2 Nitro propane-1,3-diol (18.00 mm); whereas, significantly least inhibition zone was found with Kasugamycin (13.17 mm).

Average inhibition zone was ranged from 11.37 mm (Kasugamycin) to 21.24 mm (streptocycline).

However it was significantly highest with streptocycline (21.24 mm), followed by Plantomycin (15.61 mm) and 2-Bromo-2 Nitro propane-1,3-diol (14.19 mm); whereas, significantly least inhibition zone was found with Kasugamycin (11.37 mm).

Table.1 *In vitro* efficacy of antibiotics and fungicides against *Xanthomonas axonopodis* pv. *citri*

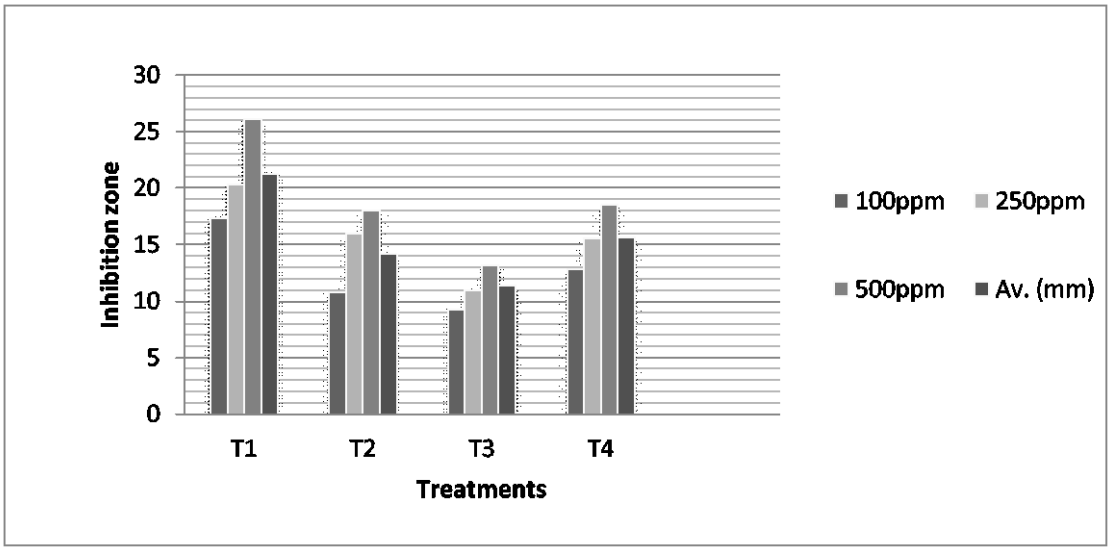
Tr. No	Treatments	Mean Inhibition zone* (mm) at			Av. (mm)
		100 ppm	250 ppm	500 ppm	
Antibiotics					
T_1	Streptocycline	17.36 **(24.61)**	20.28 **(26.74)**	26.08 **(30.70)**	21.24 **(27.35)**
T_2	2-Bromo-2 Nitro propane-1,3-diol	10.83 **(19.21)**	15.92 **(23.51)**	18.00 **(25.10)**	14.19 **(22.60)**
T_3	Kasugamycin	9.94 **(18.37)**	11.00 **(19.36)**	13.17 **(21.26)**	11.37 **(19.66)**
T_4	Plantomycin	12.81 **(20.96)**	15.53 **(23.20)**	18.50 **(25.47)**	15.61 **(23.21)**
Fungicides		**1000 ppm**	**2000 ppm**	**3000 ppm**	
T_5	Carbendazim	8.25 **(16.68)**	8.78 **(17.12)**	10.11 **(18.53)**	9.04 **(17.44)**
T_6	Bordeaux mixture	10.19 **(18.61)**	15.75 **(23.37)**	19.50 **(26.20)**	15.14 **(22.72)**
T_7	Mancozeb	16.25 **(23.75)**	18.92 **(25.77)**	20.75 **(27.09)**	18.64 **(25.53)**
T_8	Cyamoxanil + Mancozeb	11.11 **(19.46)**	13.58 **(21.62)**	17.11 **(24.42)**	13.93 **(21.83)**
T_9	Copper oxychloride	10.67 **(19.05)**	14.28 **(22.19)**	17.11 **(24.43)**	14.02 **(21.89)**
T_{10}	Control (untreated)	0.00	0.00	0.00	0.00
	S.E. +	**0.44**	**0.46**	**0.55**	-
	C.D. (P=0.01)	**1.31**	**1.38**	**1.63**	-

*Mean of three replications,
*Figures in Parenthesis are arcsine transformed values

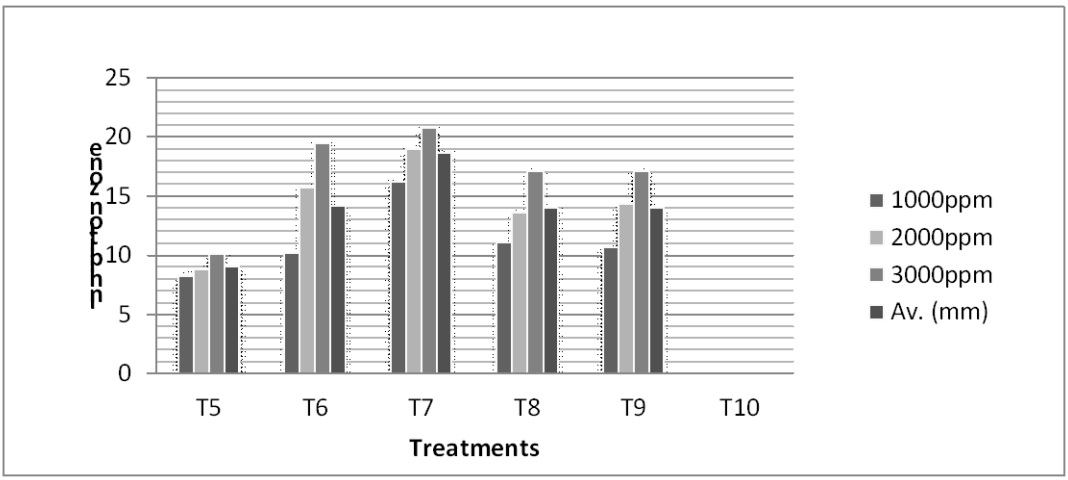
Table.2 *In vitro* efficacy of botanicals against *Xanthomonas axonopodis* pv. *citri*

Tr. No	Treatments	Inhibition zone* (mm)		
		Botanicals		
		(10%)	(20%)	Av.(%)
T_1	Ginger*(Z. officinale)*	9.83 **(18.25)**	13.83 **(21.83)**	11.83 **(20.04)**
T_2	Custard apple *(A. squimosa)*	7.42 **(15.79)**	10.42 **(18.82)**	8.92 **(17.30)**
T_3	Golden duranda *(D. erecta. L)*	7.00 **(15.33)**	10.00 **(18.43)**	8.5 **(16.88)**
T_4	Parthenium *(P.hysterophorus)*	6.08 **(14.05)**	7.58 **(15.96)**	6.83 **(15.05)**
T_5	Tulsi *(O. sanctum)*	8.08 **(16.15)**	10.92 **(19.26)**	9.5 **(17.70)**
T_6	Jakhamjudi *(T. procumbens)*	7.33 **(15.69)**	9.03 **(17.44)**	8.18 **(16.56)**
T_7	Neem *(A. indica)*	9.75 **(18.19)**	13.50 **(21.55)**	11.62 **(19.87)**
T_8	Neemseed karnel *(A. indica)*	6.33 **(14.54)**	8.83 **(17.26)**	7.58 **(15.9)**
T_9	Garlic *(A. sativum)*	9.33 **(17.78)**	12.58 **(20.77)**	10.95 **(19.27)**
T_{10}	Control (untreated)	0.00	0.00	-
	S.E. +	**0.43**	**0.53**	-
	C.D. (P=0.01)	**1.28**	**1.58**	-

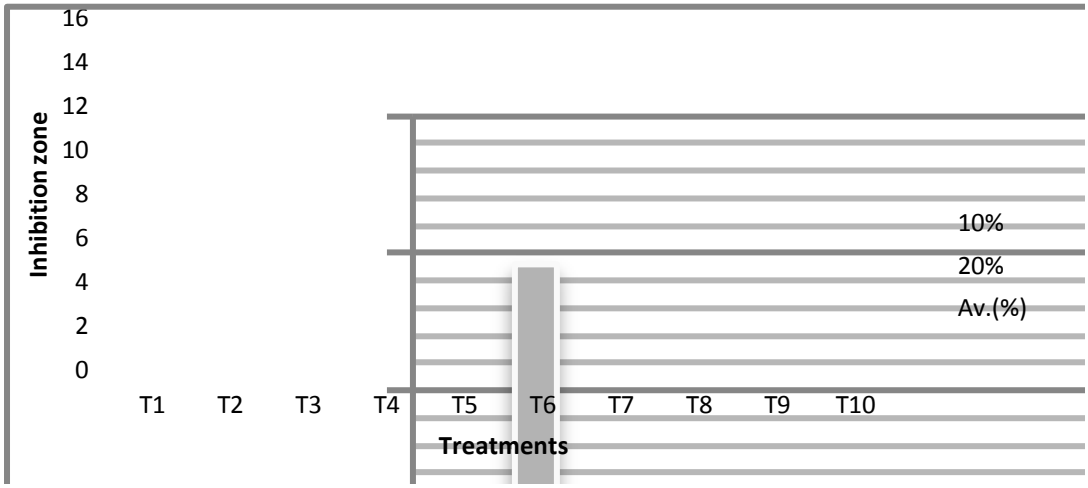
*Mean of three replications,
*Figures in Parenthesis are arcsine transformed values



***In vitro* efficacy of antibiotics**



***In vitro* efficacy of fungicides**



***In vitro* efficacy of botanicals**

Efficacy of fungicides

Results (Table 1) indicated that the antibacterial fungicides tested at various concentrations exhibited a wide range of inhibition zone in *X. axonopodis* pv. *citri*, over untreated control and it was found to be increased steadily with increase in concentrations of the test funigicides.

At 1000 ppm, bacterial growth inhibition zone was ranged from 8.25 mm (Carbendazim) to 16.25 mm (mancozeb).

However, it was significantly highest (16.25mm) with the treatment T_7 (Mancozeb), followed by T_8 (Cyamoxanil + Mancozeb) with 11.11 mm, copper oxychloride (10.67 mm), and Bordeaux mixture (10.19 mm); whereas, significantly least inhibition zone was found with Carbendazim (8.25 mm).

At 2000 ppm, bacterial growth inhibition zone was ranged from 8.78 mm (Carbendazim) to 18.92 mm (Mancozeb). However, it was significantly highest (18.92 mm) with the treatment T_7 (Mancozeb), followed by T_6 (Bordeaux mixture) with (15.75 mm), copper oxychloride (14.28 mm), and Cyamoxanil + Mancozeb (13.58 mm); whereas, significantly least inhibition zone was found with Carbendazim (8.78 mm).

At 3000 ppm, bacterial growth inhibition zone was ranged from 10.11 mm (Carbendazim) to 20.75 mm (Mancozeb). However, it was significantly highest (20.75 mm) with the treatment T_7 (Mancozeb), followed by T_6 (Bordeaux mixture) with (19.50 mm), copper oxychloride (17.11 mm), and Cyamoxanil + Mancozeb (17.11 mm); whereas, significantly least inhibition zone was found with Carbendazim (10.11 mm).

Average inhibition zone was ranged from 9.04 mm (Carbendazim) to 18.64 mm (mancozeb). However, it was significantly highest (18.64 mm) with the treatment T_7 (mancozeb), followed by T_6 (Bordeaux mixture) with (15.14 mm), copper oxychloride (14.02 mm), and Cyamoxanil + Mancozeb (13.93 mm); whereas, significantly least inhibition zone was found with Carbendazim (9.04 mm).

These results obtained in present study are in accordance with the reports of many earlier workers. The antibiotics Streptocycline (500 ppm) were reported effective against *X. axonopodis pv. citri* by Chirame and Shinde (1993) Shahid *et al.,* (2005) reported that Streptomycin sulphate, Dithane M-45 and Vitavax power significantly inhibited the multiplication of the *X. compestris* pv. *citri* at all concentrations. (each @ 0.01, 0.1 and 1% concentration). Similar results were also reported earlier by many workers. (Chakravarti and Rangarajan 1966; Desai *et al.,* 1967; Pal *et al.,* 1981; Sharma *et al.,* 1982; Krishna and Nema, 1983; Graham *et al.,* 2004; Dhakal *et al.,* 2009; Raghuwanshi *et al.,* 2013; Giri *et al.,* 2008; Mustafa *et al.,* 2015).

Efficacy of botanicals

Results (Table 2) indicated that the botanicals tested at various concentrations (each @ 10 and 20 per cent) significantly inhibited growth of *X. axonopodis* pv. *citri*, over untreated control.

At 10 per cent, inhibition zone with test aqueous phytoextracts was ranged 6.08 (*P.hysterophorus*) and 9.83 mm (*Z. officinale*); However, it was significantly highest with *Z. officinale* (9.33 mm), followed by the *A. indica* (9.75 mm), *A. sativum* (9.33 mm), *O. sanctum* (8.08 mm),

A. squimosa (7.42 mm), *T. procumbens* (7.33 mm), *D. erecta. L* (7.00mm), Neemseed karnel (6.33); whereas, significantly least inhibition zone was found with *P.Hysterophorus* (6.08).

At 20 per cent, inhibition zone with test aqueous phytoextracts was ranged 7.58(*P.hysterophorus*) and 13.83 mm (*Z. officinale*); However, it was significantly highest with *Z. officinale* (13.83 mm), followed by the *A. indica* (13.50 mm), *A. sativum* (12.58 mm), *O. sanctum* (10.92 mm), *A. squimosa* (10.42 mm), *D. erecta. L* (10.00 mm) *T. procumbens* (9.03 mm), Neemseed karnel (8.83 mm); whereas, significantly least inhibition zone was found with *P.Hysterophorus* (7.58).

Average inhibition zone was ranged from 6.83 (*P.hysterophorus*) and 11.83 mm (*Z. officinale*); However, it was significantly highest with *Z. officinale* (11.83 mm), followed by the *A. indica* (11.62 mm), *A. sativum* (10.95 mm), *O. sanctum* (9.5 mm), *A. squimosa* (8.92 mm), *D. erecta. L* (8.5 mm) *T. procumbens* (8.11 mm), Neemseed karnel (7.58); whereas, significantly least inhibition zone was found with *P.Hysterophorus* (6.83). These results of the present study are in consonance with the findings of several previous workers. Tulsi leaves extract (*Occium* spp.), Neem seed oil, Garlic bulb extract, NSKE, *Z. officinale, A. sativum* were reported antibacterial against *X. axonopodis* pv. *citri* earlier by several workers (Chanlida *et al.,* 2001; Prakash and Karmegam, 2012; Giri *et al.,* 2008; Vudhivanich, 2003; Sajid, 2013; Raju *et al.*, 2013).

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