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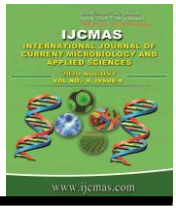

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In-Vitro Efficacy Antibiotics amongst the Isolates of *Xanthomonas axonopodis* pv. *citri*

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A B S T R A C T

Five antibiotics *viz*. Streptomycin sulphate, Streptocyclin, Chloramphenicol, Ampicillin and Rifamycin and One fungicide *viz.* Copper-Oxychloride were tested against *Xanthomonas axonopodis* pv *Citri*. in vitro. The antibiotic sensitivity against sixteen isolates was studied by paper disk method. Streptomycine sulphate (500ppm) was significantly superior over other treatments with maximum inhibition zone in all Sixteen isolates of *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). Additionally, it has enormous therapeutic values (Chaudhry *et al.*, 1992). Citrus is a member of Rutaceae family and grown in varying areas in countries with tropical or subtropical climates. The most important commercial citrus cultivars in India are the mandarin followed by sweet orange and acid lime.

Citrus is a member of Rutaceae family and grown in the world with tropical or subtropical climates. Among the major cardinal factors for low yields are the diseases. The various diseases infecting citrus are citrus canker caused by *Xanthomonas*

axonopodis pv. *citri*, gummosis (*Phytophthora* spp.), powdery mildew (*Acrosporium tiugitaninum*), anthracnose (*Colletotrichum gloeosporioides*), Tristeza (*Citrus tristeza virus*), greening (*Liberobacter candidatus asiaticum*). Citrus bacterial canker (CBC), caused by *Xanthomonas citri* pv. citri (Schaad *et al.,* 2006) is one of the most devastating diseases throughout the world that affects many kind of commercial citrus varieties. According to, Fawcett and Jenkins (1933) the origin of citrus canker is either from India, Java or some other region of Asia. It was first identified in Florida (USA) in 1915 and in India was reported from Punjab in 1942.

Citrus canker causes heavy losses and has the adverse effects on the economy. Several strategies have been adopted to manage this disease. Among them one aspect is to overcome the citrus canker through antibiotics. Antibiotics are able to control the disease but development of resistance in bacteria against these antibiotics needs investigation. Availability to farmer is another matter of concern for the policy makers. Although, lot of work has been reported in this aspect to check the effectiveness of antibiotics against *Xanthomonas axonopodis* pv. *citri* but exploration of new antibiotics at optimum concentration is the need of time. So that establishment of resistance against already available antibiotics could be avoided. Therefore the current study was proposed to evaluate the in-vitro efficacy of different antibiotics available in the local market.

Materials and Methods

Collection and isolation of diseased plant samples

Collection of diseased samples

A total of Sixteen diseased samples of acid lime infected with citrus canker were collected from fourteen agro climatic regions of India *viz*., Western Plateau Hill Region (Akola), Eastern Plateau Hill Region (Nagpur), Western Plateau Hill Region (Pune), West Cost Plane and Ghat Region (Rahuri), West Coast Plains and Ghat Region (Dapoli), Southern Plateau Hill Region (Andhra Pradesh), Upper Gangentic Plane (Uttar Pradesh), Western Himalayan Region (Uttarakhand), Western Dry land (Rajasthan), Gujarat Plane and Hill Region (Gujarat), Trans Gangentic Plane (Punjab), Southern Plateau Hill Region (Karnataka), Central Plateau and Hill Region (Madhya Pradesh), Eastern Himalayan Region (Meghalaya), Lower Gangentic Plane (West Bengal) and Eastern Coastal Plains and Hills (Odhisa) and isolated the bacterium by employing tissue isolation method on nutrient agar 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.

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.

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

Sensitivity of the sixteen isolates (*Xac*) were tested by modified paper disc assay. The derived concentration of the antibiotics and fungicides *viz*. Streptomycin sulphate,

Streptocyclin, Chloramphenicol, Ampicillin and Rifamycin and fungicide Copper Oxychloride were freshly prepared in sterile distilled water. The bacterium *Xanthomonas axonopodis* pv. *citri* was multiplied by inoculating the loopful culture in 150 ml conical flask containing 50 ml of nutrient broth medium. The inoculated flasks were incubated at 27±2^{0}C for 72 h.

The 10ml of prepared bacterial suspension of each isolate was added to conical flask containing NA, when NA media get cooled and before to solidify the medium. The medium seeded with bacterial suspension was shaken well and immediately poured in sterilized Petri plates and allowed to solidify.

The concentrations of antibiotics and fungicides were prepared. The filter paper disc (Whatman No. 42) measuring 5 mm in diameter were prepared and sterilized before use. The sterilized filter paper discs were soaked in the respective concentrations of chemicals for five minutes and transferred onto the surface of the seeded medium in Petriplates. The plates were incubated at 27±2^{0}C for 72 hrs and observed for the production of inhibition zone around the filter paper discs. The paper discs soaked in sterile distilled water were served as control. The results thus obtained were analysed statistically.

Results and Discussion

Efficacy of different Antibiotics and Fungicides against Sixteen isolates *Xanthomonas axonopodis* pv. *citri* by Paper disc method

In order to assess the efficacy of different antibiotics and fungicide against sixteen isolates of *Xanthomonas axonopodis* pv. *citri*. In the present investigation five antibiotics *viz*. Streptomycin sulphate, Streptocyclin, Chloramphenicol, Ampicillin and Rifamycin and One fungicide viz. Copper Oxychloride were used against *Xanthomonas axonopodis* pv. *citri*.@100, 250 and 500 ppm concentration by paper disk method. Result indicated that the antibiotics and fungicides at various concentrations were significantly inhibited the growth of *Xanthomonas axonopodis* pv. *citri* over untreated control (Table no. 1 a, b, c and d).

At 100 ppm concentration the bacterial inhibition zone was ranged from 00.00 mm (Rifamycin) to 11.67 mm (Streptomycin sulphate). The significantly highest inhibition zone of Xac3 isolates with Streptomycin sulphate (11.67 mm) and it was followed by Streptocyclin 9.33 mm for same Isolates where as no inhibition zone recorded in all isolates of *Xanthomonas axonopodis* pv. *citri*. with Rifamycin antibiotic (00.00 mm) an control.

At 250 ppm concentration the bacterial inhibition zone was ranged from 00.00 mm (Rifamycin) to 16.00 mm (Streptomycin sulphate). The highest inhibition zone of xac3 isolates with Streptomycin sulphate to 16.00 mm and it was followed by Streptomycin sulphate 13.00 mm for Xac1 isolates.

At 500 ppm concentration the bacterial inhibition zone was ranged from 00.00 mm (Rifamycin) to 18.67 mm (Streptomycin sulphate). The significantly highest inhibition zone of Xac4 isolates with Streptomycin sulphate 18.67 mm and it was followed by Streptomycin sulphate 17.33 mm for Xac1 and Xac3 Isolates.

Among the all sixteen isolates tested with antibiotics and one fungicides Xac4 (18.67 mm) and Xac3 (16.00 mm) showed maximum zone inhibition at 250 and 500 ppm concentration. However least zone of inhibition was recorded in Rifamycin.

Table.1(a) Antibiotic sensitivity against *Xanthomonas axonopodis* pv. *citri* isolates by paper disk technique

Tre. No.	Antibiotics	Xac1 (Akola)			Xac2 (Nagpur)			Xac3 (Pune)			Xac4 (Rahuri)		
		100	**250**	**500**	**100**	**250**	**500**	**100**	**250**	**500**	**100**	**250**	**500**
T_1	Streptomycin sulphate	11.11	13.33	17.33	7.33	9.67	11.33	11.67	14.00	17.33	7.67	9.67	18.67
T_2	Streptocycline	5.33	7.33	8.67	4.67	5.33	7.33	9.33	11.67	13.33	4.00	5.33	7.33
T_3	Copper oxychloride	4.33	7.67	8.33	5.33	6.00	7.33	4.34	5.67	6.00	5.67	6.00	7.67
T_4	Chloramphenicol	4.33	5.33	8.33	5.67	7.33	8.67	6.67	8.67	11.00	4.67	6.67	9.67
T_5	Ampicillin	3.33	5.67	10.33	0.00	0.00	8.33	0.00	5.33	7.33	5.33	7.67	9.00
T_6	Rifamycin	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T_7	Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	F Test	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
	SE± (M)	**0.47**	**0.50**	**0.40**	**0.36**	**0.44**	**0.50**	**0.31**	**0.47**	**0.44**	**0.36**	**0.47**	**0.47**
	CD	**1.40**	**1.50**	**1.19**	**1.06**	**1.30**	**1.50**	**0.92**	**1.40**	**1.30**	**1.06**	**1.40**	**1.40**

Table.1(b) Antibiotic sensitivity against *Xanthomonas axonopodis* pv. *citri* isolates by paper disk technique

Tre. No.	Antibiotics	Xac5 (Dapoli)			Xac6 (A. P.)			Xac7 (U.P)			Xac8 (UK)		
		100	**250**	**500**	**100**	**250**	**500**	**100**	**250**	**500**	**100**	**250**	**500**
T_1	Streptomycin sulphate	5.33	6.00	9.33	6.67	8.67	10.33	10.00	10.67	12.67	6.67	8.33	10.67
T_2	Streptocycline	7.33	9.67	11.67	7.33	10.00	11.00	5.33	10.33	12.33	4.33	6.00	6.33
T_3	Copper oxychloride	5.67	6.67	7.67	6.67	8.67	9.33	0.00	5.67	7.00	0.00	2.67	3.33
T_4	Chloramphenicol	4.67	6.67	8.33	4.33	6.67	7.67	4.67	8.67	11.33	0.00	4.67	7.00
T_5	Ampicillin	0.00	4.33	6.33	4.00	6.33	9.67	4.67	6.67	9.33	4.33	5.33	8.00
T_6	Rifamycin	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T_7	Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	F Test	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
	SE± (M)	**0.36**	**0.47**	**0.40**	**0.36**	**0.47**	**0.47**	**0.44**	**0.40**	**0.56**	**0.31**	**0.36**	**0.53**
	CD	**1.06**	**1.40**	**1.19**	**1.06**	**1.40**	**1.40**	**1.30**	**1.19**	**1.68**	**0.92**	**1.06**	**1.59**

Table.1(c) Antibiotic sensitivity against *Xanthomonas axonopodis* pv. *citri* isolates by paper disk technique

Tre. No.	Antibiotics	Xac9 (Rajasthan)			Xac10 (Gujrat)			Xac11 (Punjab)			Xac12 (Karnataka)		
		100	**250**	**500**	**100**	**250**	**500**	**100**	**250**	**500**	**100**	**250**	**500**
T_1	Streptomycin sulphate	7.33	9.67	11.33	8.00	9.67	12.67	6.67	8.67	10.67	8.33	10.33	11.33
T_2	Streptocycline	8.67	10.67	12.67	7.33	9.67	11.33	6.67	8.67	10.67	4.33	6.67	8.33
T_3	Copper oxychloride	0.00	3.33	4.33	0.00	3.67	4.35	0.00	1.20	1.98	0.00	2.65	3.56
T_4	Chloramphenicol	4.33	5.67	6.33	5.67	8.67	11.00	5.67	9.67	12.00	5.33	7.67	8.67
T_5	Ampicillin	0.00	4.33	8.00	0.00	0.00	8.33	0.00	0.00	8.33	0.00	0.00	8.67
T_6	Rifamycin	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T_7	Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	F Test	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
	SE± (M)	**0.36**	**0.40**	**1.78**	**0.40**	**0.36**	**0.47**	**0.31**	**0.44**	**0.56**	**0.36**	**0.36**	**0.50**
	CD	**1.06**	**1.19**	**5.30**	**1.19**	**1.06**	**1.40**	**0.92**	**1.30**	**1.68**	**1.06**	**1.06**	**1.50**

Table.1(d) Antibiotic sensitivity against *Xanthomonas axonopodis* pv. *citri* isolates by paper disk technique

Tre. No.	Antibiotics	Xac13 (M. P.)			Xac14 (Meghalaya)			Xac15 (West Bengal)			Xac16 (Odhisa)		
		100	**250**	**500**	**100**	**250**	**500**	**100**	**250**	**500**	**100**	**250**	**500**
T_1	Streptomycin sulphate	7.33	8.67	10.67	10.67	12.67	14.33	5.33	7.67	9.00	5.33	7.00	8.00
T_2	Streptocycline	8.67	11.33	14.33	9.33	11.33	13.33	8.00	9.67	11.33	7.00	9.67	12.67
T_3	Copper oxychloride	0.00	1.75	2.23	0.00	3.34	3.98	0.00	3.56	4.89	5.33	6.74	7.53
T_4	Chloramphenicol	6.67	10.33	13.00	7.33	11.00	14.67	8.67	12.00	14.33	8.67	12.00	15.33
T_5	Ampicillin	5.67	8.00	11.67	0.00	4.33	6.67	4.33	6.67	11.33	0.00	5.00	8.00
T_6	Rifamycin	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T_7	Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	F Test	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
	SE± (M)	**0.40**	**0.71**	**0.47**	**0.31**	**0.71**	**0.40**	**0.53**	**0.47**	**0.36**	**0.44**	**0.73**	**0.62**
	CD	**1.19**	**2.12**	**1.40**	**0.92**	**2.12**	**1.19**	**1.59**	**1.40**	**1.06**	**1.30**	**2.19**	**1.84**

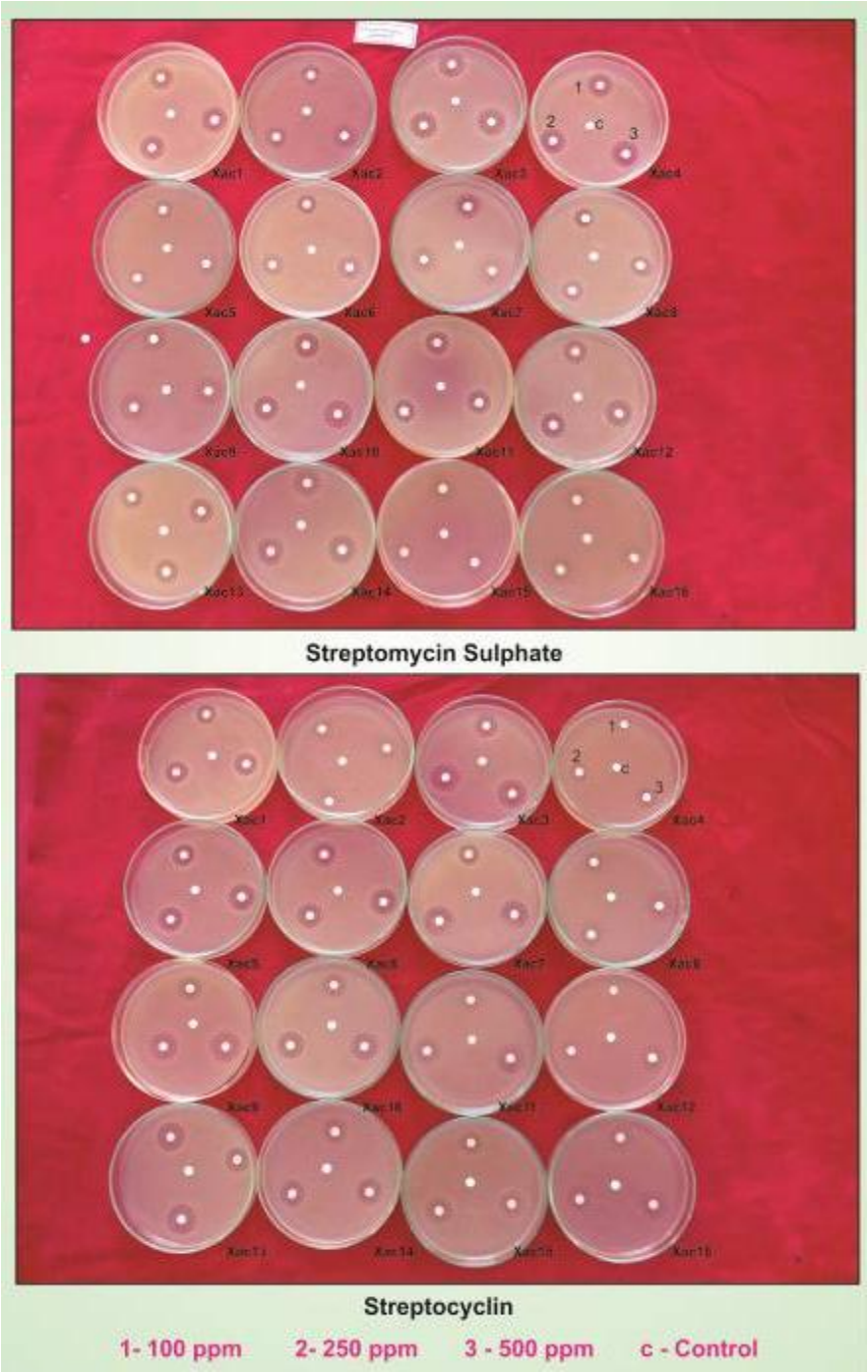

Plate.1 (a) Antibiotic sensitivity against *Xanthomonas axonopodis* pv. Citri isolates by paper disk technique

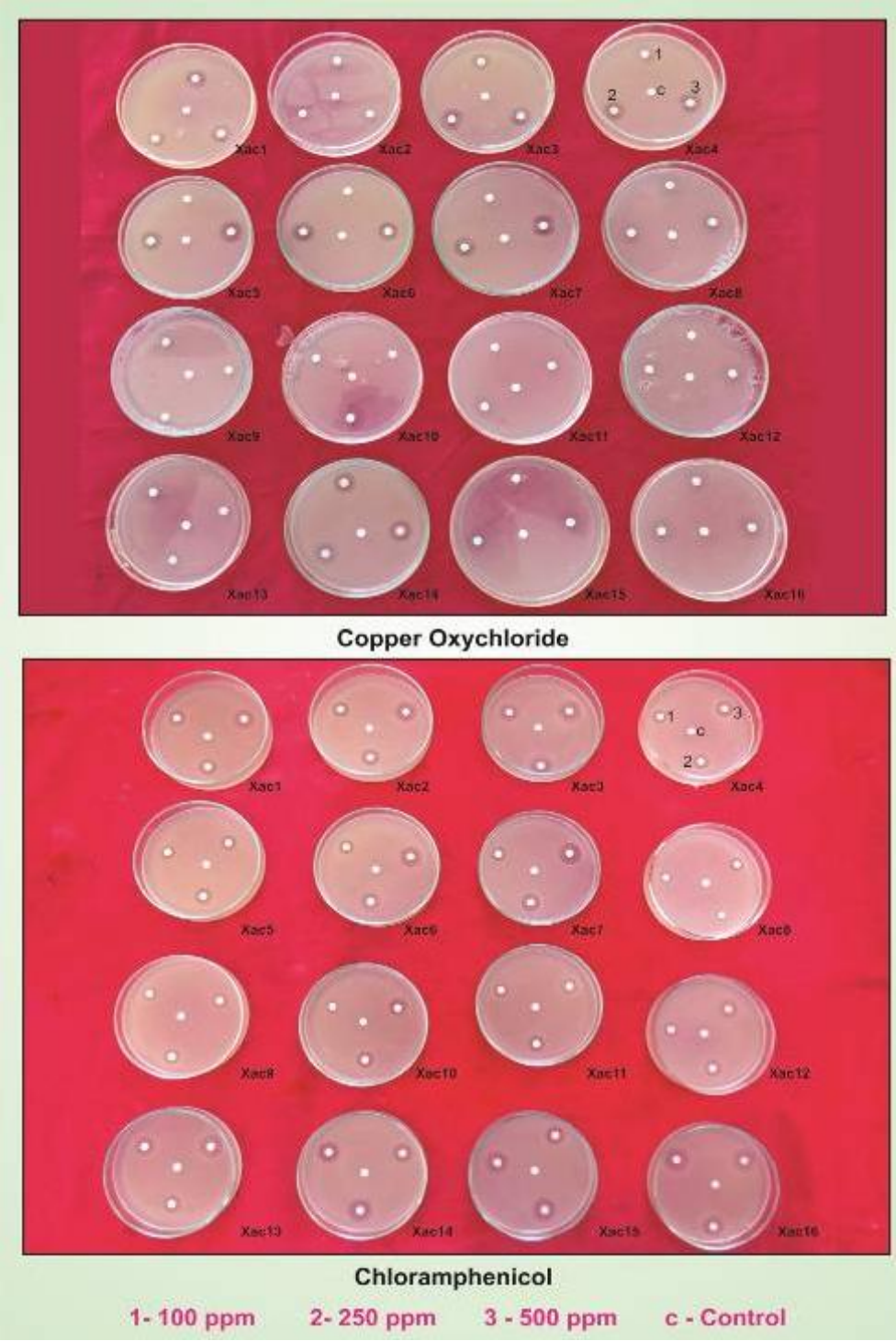

Plate.1 (b) Antibiotic sensitivity against *Xanthomonas axonopodis* pv. citri isolates by paper disk technique

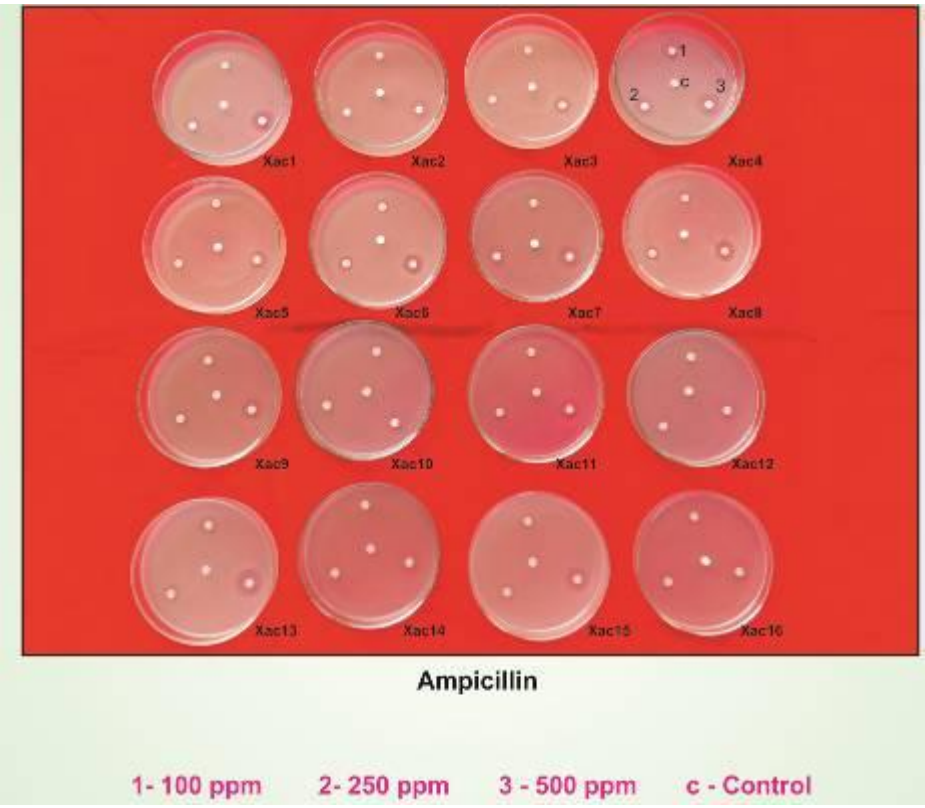

Plate.1 (c) Antibiotic sensitivity against *Xanthomonas axonopodis* pv. citri isolates by paper disk technique

The result are in confirmation of Valenchia *et al.,* (2004) they collected 123 Isolates of *Xanthomonas axonopodis* pv. *dieffenbachiae* (Xad) in Los Banos and tested in-vitro. Among the isolates, 33 were found resistant to 500 ppm streptomycin; 29 were found resistant to 200 ppm, 17 to 500 ppm, 16 to 1,000 and 12 to 2,000 ppm streptomycin.

Das (2005) studied efficacy of different chemicals against different isolates of *Xanthomonas axonopodis* pv.*citri* and found that COC (0.3%) + streptomycin sulphate 100 ppm was found more effective in reducing citrus canker in vitro by paper disc and turbidiometrical methods.

Maher *et al.,* (2005) tested different toxicanta viz. Streptomycin sulphate, Dithane M-45, Agrimycin – 100, Vitavax, Benlate and Cobox at 0.01, 0.1 and 1% concentration against *Xanthomonas axonopodis* pv. *citri* out of these Streptomycin sulphate was found to be the most effective among the toxicants used.

Raghuwanshi *et al.,* (2013) used different sets of treatments to evaluate in vitro efficacy of *Xanthomonas axonopodis* pv *punicae* at different concentrations of chemicals. The most variable response was observed at Streptocycline 250 ppm ranging from 58.56 to 99.19 % inhibition of bacterium.

Islam *et al.,* (2014) studied in vitro antibiotic sensitivity pattern of bacterium responsible for citrus canker. All the isolates were tested for antibiotic sensitivity against 5 commonly used antibiotics namely, cefotaxime (30 µg conc.), bacitracin (10 µg conc.), chloramphenicol (30 µg conc.), streptomycin (10 µg conc.) and gentamycin (10 µg conc.). The bacterium showed 55.5% sensitivity to streptomycin at 10 µg conc.

Abhang *et al.,* (2018) studied the different bioagents, botanicals and chemicals against Xanthomonas axonopodis pv. citri and revealed that the Copper oxychloride (0.2%) + streptomycin sulphate (200 ppm) was significantly inhibit the growth of pathogen and in botanicals and bioagents neem seed kernel extract (5%) was effective in reducing the growth of bacteria with 0.446 OD at 96 h followed by Pseudomonas flourescence 1x108 cell and Bacillus subtilis 1x108cell with 0.506 and 0.486 OD, respectively. Jadhav *et al.,* (2018) studied on three antibiotics, three antibacterial fungicides and three botanicals for the management of bacterial canker disease of kagzi lime caused by *Xanthomonas axonopodis* pv. *citri.* (Pot culture). In these antibiotics, streptocycline was found most effective with lowest PDI mean 33.77 per cent.

The present study confirms the findings of the researches that the different antibiotics and fungicides with their different doses behaved differently to the isolates of the different agro climatic regions. A few of them showed no inhibition to the different antibiotics and fungicidal concentrations, this could be due to presence of some by pass mechanisms to avoid the toxicity of the fungicides or antibiotics or harboring the plasmid that offers the resistance against these antibiotics and fungicides that needs the further investigation at molecular level.

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