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Evaluation of antibiotics, antibacterial chemicals and bio-agents against citrus canker caused by *Xanthomonas axonopodis* pv. *citri* (Hasse)

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ABSTRACT

Six antibiotics, two antibacterial chemicals and five bio-agents were evaluated by *in vitro* against *Xanthomonas axonopodis* pv. *citri* (Hasse). Among the different antibiotics and antibacterial chemicals, Streptocycline (10.84 mm) and copper oxychloride (7.50 mm) showed maximum inhibition zone followed by K cycline (9.68 mm). Among the bio-agents, *Bacillus subtilis* was effective with the inhibition zone of (16.16 mm) followed by *Pseudomonas fluorescens* (14.63 mm). The fungal bio control agents *viz.*, *Trichoderma viride* and *Trichoderma harzianum* were found totally in effective against the pathogen.

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INTRODUCTION

Citrus is a delectable, juicy and seedless fruit having great nutritional significance (Khan *et al.*, 1992). Additionally, it possesses enormous therapeutic qualities (Chaudhary *et al.*, 1992). Citrus canker is caused by the bacteriumk "*Xanthomonas axonopodis* pv. *citri*" that is probably the worst enemy to citrus plants. 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. As such, disease severity on susceptible variety results in defoliation, dieback, premature fruit drop and blemished fruit, which consequently decrease fruit production and market value.

MATERIAL AND METHODS

A set comprising five commercially available antibiotics and two antibacterial chemicals (each at two concentrations) and five bio agents were evaluated for their efficacy against the growth of *X. axonopodis* pv. *citri* by inhibition zone assay method.

The bacterium was multiplied by inoculating the culture into the 20 ml of nutrient broth taken in 'Erleyenmayers' flask. The inoculated flasks were incubated at 30^{0}C for 72 hours. The bacterial suspension was then seeded to the lukewarm nutrient agar medium (1000 ml). The seeded medium was poured into the sterilized Petri plates and plates were allowed to solidify.

Treatments	Chemicals	Concentrations (%)	
T_1	Bacterinashak	0.05	0.1
T_2	Streptocycline	0.05	0.1
T_3	Bacterinol-100	0.05	0.1
T_4	Plantomycin	0.05	0.1
T_5	K cycline	0.05	0.1
T_6	Copper oxy chloride	0.05	0.1
T_7	Copper hydroxide	0.05	0.1
T_8	Control		

The solution of bactericides was prepared at different concentrations as mentioned in the list. The filter paper discs (Whatman No. 42) measuring 5 mm in diameter were soaked in the respective chemical solution for 5 minutes and transferred onto the surface of the seeded medium in Petri plates. The inoculated plates were kept in the refrigerator at 50^{0}C for 4 hours to allow the diffusion of chemical into the medium. Then plates were incubated at 30^{0}C for 72 hours and observed for the production of inhibition zone around the filter paper discs. The results obtained were analysed statistically.

RESULTS AND DISCUSSION

Results revealed that, out of seven treated chemicals, streptocycline was found significantly superior in inhibiting the growth of the pathogen by inhibition zone of 10.84 mm. K-cycline (9.68 mm) and COC (7.50 mm) were the best next best effective chemicals differing significantly each other. The other treatments *viz*., Tagmycin (7.16 mm), Bacterinashak (6.33 mm), Plantomycin (5.64 mm) and kocide (4.51 mm) were found moderately effective and Bionol-100 was found significantly least effective with an inhibition zone of (3.52 mm) (Table 1 and Fig. 1).

Between the concentrations, efficacy of the bactericide was significant from lower to higher concentration with greater efficacy at higher concentrations.

Interaction effect among the antibiotics and concentration indicated that, streptocycline and K-cycline @0.1 per cent concentration were highly effective with an inhibition zone of 18.96 mm and 17.86 mm, respectively followed by copper oxychloride @ 1 per cent concentration (13.66 mm).

The present findings are in agreement with the report of Chakravarti and Rangarajan (1966), who have

Table 1 : Efficacy of antibiotics and antibacterial chemicals against the growth of *X. axonopodis* pv. *citri* under *in vitro*

Sr. No.	Antibiotics	Inhibition zone (mm) Concentration (%)		
		0.10	0.05	Mean
1.	Streptocycline (HAL)	18.96 (4.47)*	13.55 (3.81)	10.84 (3.44)
2.	K cycline	17.86 (4.34)	11.18 (3.27)	9.68 (3.27)
3.	Plantomycin	10.15 (3.34)	6.78 (2.58)	5.64 (2.58)
4.	Bacterinashak	11.20 (3.49)	7.78 (2.71)	6.33 (2.71)
5.	Bionol-100	6.89 (2.81)	3.68 (2.13)	3.52 (2.13)
6.	Tagmycin	12.62 (3.69)	8.85 (2.86)	7.16 (2.86)
Antibacterial chemicals				
7.	COC	13.66 (3.83)	8.85 (2.92)	7.50 (2.92)
8.	Kocide	7.82 (2.87)	5.72 (2.35)	4.51 (2.35)
9.	Control	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
Mean		11.01 (3.47)	7.37 (2.89)	6.13 (2.67)
Source		S.E.±	C.D. (P=0.01)	
Treatments (A)		0.272	1.046	
Concentration (B)		0.128	0.493	
A x B		0.384	1.480	

* Figures in the parentheses are $\sqrt{x}$ + 1 transformed values

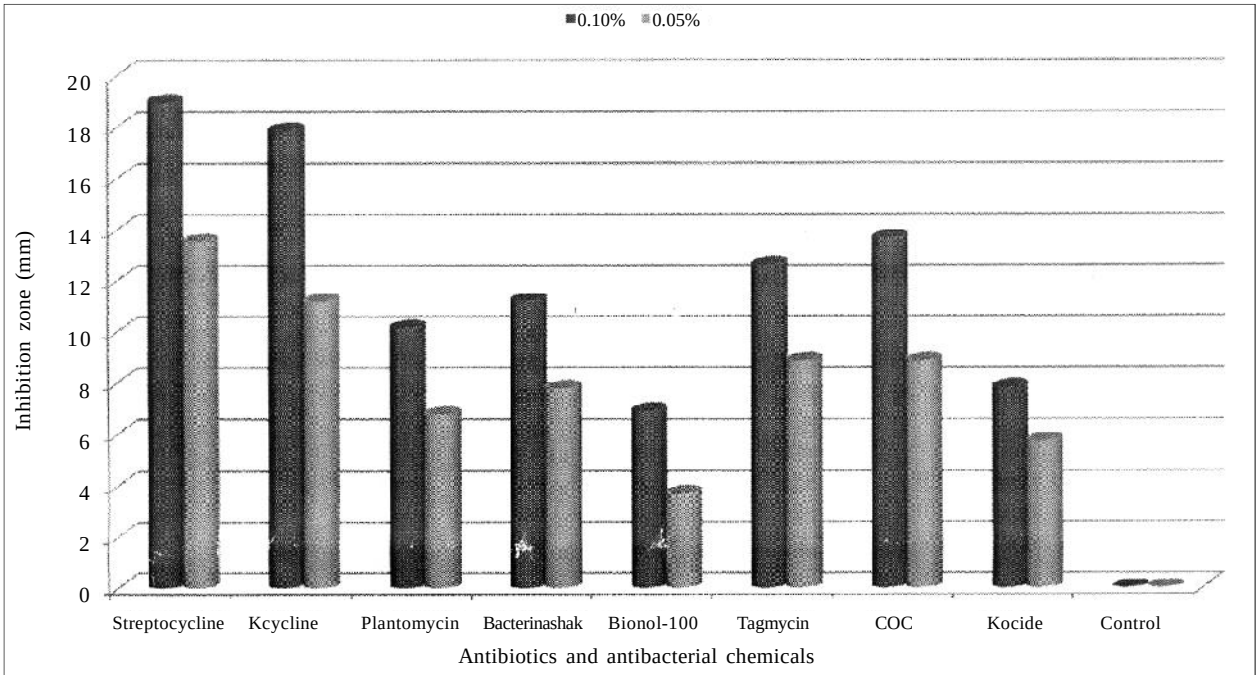

Fig. 1 : Efficacy of antibiotics and antibacterial chemicals against *X. axonopodis* pv. citri under *in vitro*

Table 2 : Efficacy of bio control agents against growth of *X. axonopodis* pv. citri under *in vitro*

Sr. No.	Bio-agents	Inhibition zone (mm) Mean
1.	*Pseudomonas fluorescens* (RP-41)	14.63 (3.95)*
2.	*Pseudomonas putida* (RP-56)	7.42 (2.90)
3.	*Bacillus subtilis*	16.16 (4.14)
4.	*Trichoderma viride* (TV-1)	0 (1.00)
5.	*Trichoderma harzianum* (TH-1)	0 (1.00)
6.	Control	0 (1.00)
	S.E.±	0.447
	C.D. (P=0.01)	1.930

* Figures in the parentheses are $\sqrt{x + 1}$ transformed values

obtained the superior efficacy of streptocycline on seven species of *Xanthomonas,* six species of *Erwinia* and one each of *Pseudomonas, Corynebacterium* and *Agrobacterium*. Desai *et al*. (1967) reported the maximum inhibition of *Xanthomonas* and *Pseudomonas* by streptocycline at 250 ppm.

Sharma *et al.* (1981) reported that, the combination of streptocycline and copper sulphate was most effective in inhibiting the growth of *Xanthomonas vesicatoria* as assessed by *in vitro* paper disc method.

Among the five biocontrol agents tried, *Bacillus subtilis*, *Pseudomonas fluorescens* and *Pseudomonas putida* were found was found significantly effective in reducing the growth of the pathogen. These two bio control agents recorded an on par inhibition zone of 16.16 mm and 14.63 mm, respectively. The next best effective bio control agent was *Pseudomonas putida* (7.42 mm diameter) (Table 2 and Fig. 2).

Use of biocontrol agents in the management of plant diseases is an age old practice and effectiveness of bacterial biocontrol agents against various crop diseases was reported by earlier workers. Kalita *et al.* (1996) reported the reduction of citrus canker by the spray application of *Pseudomonas fluorescens*, *Bacillus subtilis* and *Bacillus polymixa*. They recorded the least canker incidence with the treatment by *Bacillus subtilis*.

Unnamalia and Gnanamanikam (1984) reported the inhibiting effect of *Pseudomonas fluorescens* on the

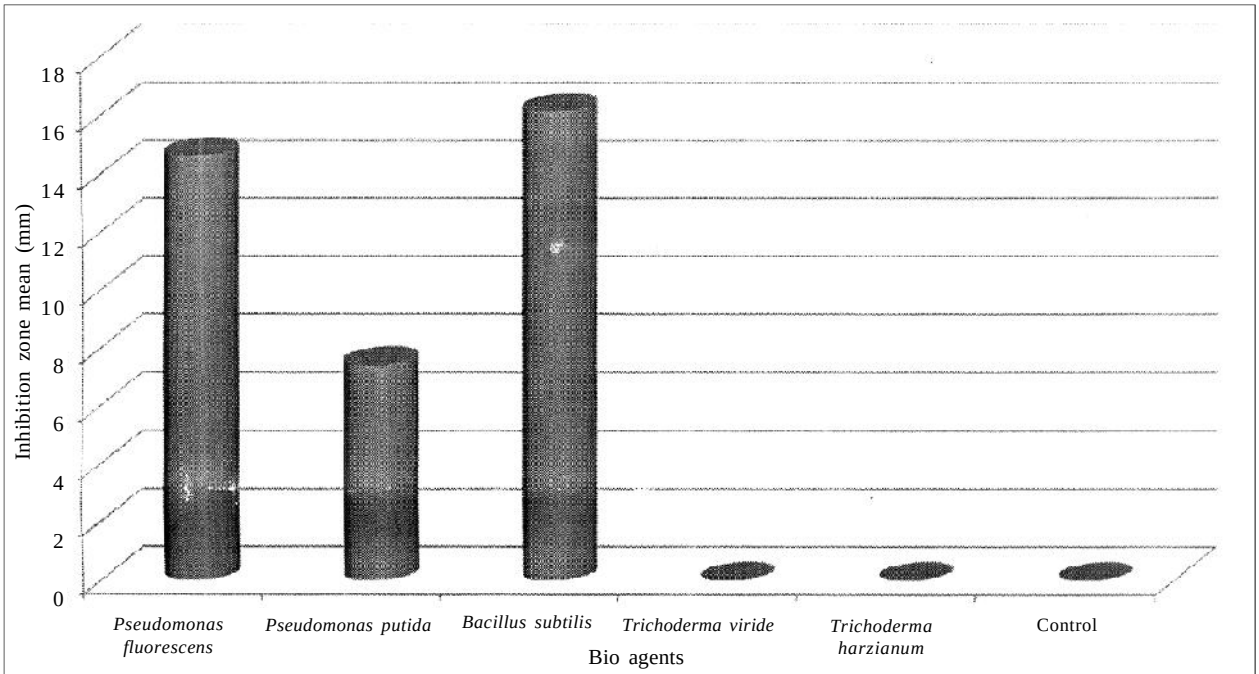

Fig. 2 : Efficacy of bio control agents against *X. axonopodis* pv. *citri* under *in vitro*

growth the *Xanthomonas citri*.

Giri *et al.* (2008) studied efficacy of bio agents *viz.*, *Trichoderma harzianum, Pseudomonas fluorescens* and *Aspergillus niger* against citrus canker. Khodakaramian *et al.* (2008) showed the antagonistic activity of all the selected *Pseudomonas* bacterial strains on *Xanthomons citri*. The highest inhibition zone belonged to *P. fluorescens* (strain 15) and *Pseudomonas putida* (strain 1). Two strains of *Pseudomonas fluorescens* (16 and 19) showed the highest antagonistic activity against *Xanthomons citri* and showed significant reduction of the leaf spots under glass house conditions. The results of present investigations also emphasized the superior efficacy of *Bacillus subtilis* and *Pseudomonas fluorescens* in reducing the growth of pathogenic bacterium *X. axonopodis* pv. *citri*.

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