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In vitro evaluation of antibiotics and antibacterial chemicals against *Ralstonia solanacearum* infecting bacterial wilt in chilli

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Abstract

An experiment was conducted to find out the effective antibacterial chemicals against the growth of *Ralstonia solanacearum* causing bacterial wilt of chilli under *in vitro* conditions. Among the five antibiotics (at concentrations each @ 400 and 500 ppm) and two antibacterial fungicides (at concentrations each @ 1500 and 2000 ppm) tested significantly inhibited growth of *R. solanacearum*, over untreated control. Among the five antibiotic tested average inhibition zone was ranged from 11.20 mm to 21.10 mm. However, it was significantly highest with streptocycline (21.10 mm), followed by gentamycin (18.11 mm), tetracycline (16.75 mm) and cephalexin (14.96 mm); whereas, it was significantly least with neomycine (11.20 mm). Among the two antibacterial fungicides tested average inhibition zone was ranged from 11.84 mm to 12.84 mm. However, it was significantly highest (12.84 mm) with the treatment T₆ (copper oxychloride), followed by T₇ (Copper hydroxide) with 11.84 mm.

Keywords: Chilli, Ralstonia solanacearum, inhibition, antibiotics, antibacterial fungicides

Introduction

Chilli (Capsicum annuum L.) is most widely used as universal spice of India. It belongs to family Solanaceae. Chilli is grown in both tropical and sub-tropical climate as it comes up well in warm humid climate with an optimum temperature of 20 to 25°C. It is preferred for its pungency, spicy taste besides the appealing color it imparts to the food. Chilli is economically very important and valuable crop throughout the world. Among all the pathogen the bacterial wilt caused by Ralstonia solanacearum is the most lethal disease of chilli. Bacterial wilt of chilli has five different races, each infecting different plant species. R. solanacearum stains are grouped into six biovars based on the biochemical tests. Chilli bacterial wilt mostly caused by strains belongs to race 1 and biovar 3. Race 1 has wide host range including solanaceous vegetables like brinjal, tomato and tobacco, while race 2 infect banana, race 3 infect potato, race 4 infects ginger and race 5 infects mulberry (Buddenhagen, 1986). This bacterium has wide host range of 450 plant species belonging to more than 54 families. Bacterial wilt incidence is mostly found in the acidic soils (soil pH < 7.0) and in the coastal humid areas. High moisture and temperature are favorable for disease development. Bacterial wilt caused by R. solanacearum is a highly devastating disease of solanaceous crops causing significant yield reduction from 10 to 90% (Yabuuchi et al, 1954). Typical symptoms on chilli plants of the bacterial will observed were lower leaves turn pale yellow and los their turgidity, followed by drooping of the leaves and sudden wilting of chilli plants, brown discoloration of vascular tissues of infected stem of chilli plants.

The aim of present investigation was to study the effect of antibiotics and antibacterial fungicides on the growth of *R. solanacearum* under *in vitro* conditions.

Materials and Methods: *In vitro* evaluation of antibacterial chemicals, five antibiotics (each @ 400 and 500ppm) and two fungicides (each @ 1500 and 2000 ppm) by inhibiton zone assay method were evaluated *in vitro* against *R. solanacearum*. The mass multiplied broth culture of the test bacterium $(2 \times 10^8 \text{ cfu/ml})$ was seeded to autoclaved Nutrient agar medium, mixed thoroughly and poured into sterilized glass Petri plates allowed to solidify. The solutions of the desired concentrations of the test antibiotics and fungicides were prepared separately. The filter paper discs (Whatman No. 42) of 5 mm in diameter were soaked separately in the respective chemical solutions for 5-10 minutes and transformed in center onto the

solidified bacterium seeded NA medium in Petri plates. The inoculated plates were kept in the refrigerator at 4 ^oC for 4 hours to allow diffusion of the chemical into medium. The untreated control plate containing with the test bacterium seeded NA and inoculated with filter paper disc soaked in distilled water was also maintained then the plates were incubated at 28 ^oC for 48 hours and observed for the production of inhibition zone around filter paper discs.

Results and Discussion: *In vitro* evaluation of antibacterial chemicals against *R. solanacearum* present investigation was carried out to evaluate antibacterial chemicals to find out their effectiveness against the growth of *R. solanacearum* under *in vitro* condition and the results were presented in Table 1. Total five antibiotics viz., Sreptocycline, Tetracycline Cephalexin, Gentamycin, Neomycine and three antibacterial fungicides viz., Copper oxy chloride and Copper hydroxide were evaluated *in vitro* by inhibition zone assay method against *R. solanacearum*.

Results (Table 1) revaled that the antibiotics tested at various concentrations (each @ 400 and 500 ppm) significantly inhibited growth of *R. solanacearum*, over untreated control. (PLATE I and Fig. 1). At 400 ppm, bacterial inhibition zone was ranged from 10.33 mm (neomycine) to 18.28 mm (streptocycline). However it was significantly highest with streptocycline (18.28 mm), followed by gentamycine (16.09 mm), tetracycline (15.17 mm) and cephalexin (13.60 mm), later three antibiotics were found at par in succession; whereas, significantly least inhibition zone was found with neomycine (10.33 mm).

At 500 ppm, bacterial growth inhibition zone was ranged from 12.08 mm (Neomycine) to 23.92 mm (streptocycline). However, it was significantly highest with streptocycline (23.92 mm), followed by gentamycine (20.13 mm) tetracycline (18.33 mm) and cephalexin (16.33 mm), whereas it was least with neomycine (12.08 mm).(PLATE II and Fig 1).

Average inhibition zone was ranged from 11.20 mm to 21.10 mm. However, it was significantly highest with streptocycline (21.10 mm), followed by gentamycin (18.11 mm), tetracycline (16.75 mm) and cephalexin (14.96 mm); whereas, it was significantly least with neomycine (11.20 mm).

Effect antibacterial fungicides

Results (Table 1) revealed that the antibacterial fungicides tested at various concentrations exhibited a wide range of inhibition zone in *R. solanacearum*, over untreated control and it was found to be increased steadily with increase in concentrations of the test fungicides (PLATE I and II, Fig. A & B). At 1500 ppm, bacterial growth inhibition zone was ranged from 10.53mm (Copper hydroxide) to 11.19 mm (Copper oxychloride). However, it was significantly highest (11.19 mm) with the treatment T_6 (copper oxychloride), followed by T_7 (Copper hydroxide) with10.53 mm.

At 2000 ppm, bacterial growth inhibition was ranged from 13.16 mm (Copper hydroxide) to 14.50 mm (copper oxychloride). However, it was significantly highest (14.50), with the treatment T_6 (copper oxychloride), followed by T_7 (Copper hydroxide) with 13.16 mm.

Tr. No.	Treatment	Mean inhibition zone (mm)*		
		400ppm	500ppm	Av. (mm)
		Antibiotics		
T ₁	Streptocycline	18.28	23.92	21.10
T2	Tetracycline	15.17	18.33	16.75
T3	Neomycine	10.33	12.08	11.20
T 4	Cephalexin	13.60	16.33	14.96
T ₅	Gentamycine	16.09	20.13	18.11
Antibacterial fungicides		1500ppm	2000ppm	
T ₆	Copper oxychloride	11.19	14.50	12.84
T ₇	Copper hydroxide	10.53	13.16	11.84
T8	Control	0.00	0.00	0.00
	S.E.±	0.71	0.83	0.77
	C.D. (P=0.01)	2.10	2.44	2.27

Table A: In vitro efficacy of antibiotics and antibacterial fungicides against R. solanacearum

* Mean of three replications

Average inhibition zone was ranged from 11.84 mm to12.84 mm. However, it was significantly highest (12.84 mm) with the treatment T_6 (copper oxychloride), followed by T_7 (Copper hydroxide) with 11.84 mm. Thus, all the antibiotics and antibacterial fungicides tested were found bacteriostatic

against *R. solanacearum* and significantly inhibited its colony growth. However, the antibiotics viz., streptocycline, followed by Gentamycine and Tetracycline; the fungicide copper oxychloride was found most promising against the bacterium *R. solanacearum*.



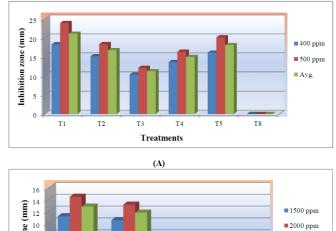
Tr. No	Truman	Tr. No	Treatrots
Ι	streptoccline	5	gentamycine
2	tetracycline	6	Copper oxychloride
3	neomycine	7	Copper hydroxide
4	cephalexin	8	azoxystrobin

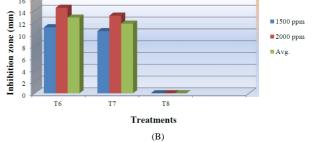
Plate I: *In vitro* efficacy of antibiotics and fungicides against *R. solanacearum* teach antibiotics @ 400 ppm and antibacterial fungicides @ 1500 ppm.



Tr. No	Treameats	Tr. No	Trammel	
1	Streprocychae	5	gentanmycine	
2	Tetracycline	6	Copper oxychloride	
3	neomycine	7	Copper hydroxide	
4	Cephxtexut	IS	Azoxyorobur	

Plate II: In vitro effcacy of antibiotics and fungicides against Ralstonia solanacearum (each antibiotics @ 500 ppm and antibacterial fungicides @ 2000 ppm)





Tr. No	Treatments	Tr. No	Treatments	
T_1	Streptocycline	T5	Gentantycine	
T_2	Tetracycline	T ₆	Copper oxychloride	
T3	Neomycine	T 7	Copper hydroxide	
T 4	Cephalexin	T8	Control	

Fig 1: *In vitro* efficacy of various antibiotics (A) and antibacterial fungicides (B) against *R. solanacearum*

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