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## *In vitro* evaluation of antibiotics against *Ralstonia solanacearum*

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### Abstract

Tomato (*Solanum lycopersicum* L.) is one of the most prevalent vegetable food crops in the world, *Ralstonia solanacearum* causing vegetable bacterial wilt in tomato is the most destructive pathogen across the world, in the present investigation five antibacterial chemicals (three antibiotics, one fungicide and one plant derived antibacterial chemical) were evaluated for their efficacy against bacterial wilt pathogen *in vitro* condition. Among all the treatments at different concentrations streptomycin 500 ppm produced highest inhibition zone of 20.00 mm, followed by 2-Bromo-2-Nitropropane-1,3-diol (bacterinash) 500 ppm showed 18.40 mm inhibition zone whereas streptomycin 300 and 150 ppm produced inhibition zone of 17.60 and 15.80 mm respectively, after that 2-Bromo-2-Nitropropane-1,3-diol produce a moderate effect with 15.40 and 13.40 mm at 300 and 150 ppm concentrations respectively, followed by copper oxychloride produce 8.80 mm and bacterisan produce 7.40 mm inhibition zone at 500 ppm concentrations. However at 150 and 300 ppm concentrations, both bacterisan and copper oxychloride and all concentrations of validamycin did not form any inhibition zone.

**Keywords:** *Ralstonia solanacearum*, Antibiotics, tomato, *Solanum lycopersicum* L., Wilt

### Introduction

Tomato (*Solanum lycopersicum* L.), (2n=24) is the most prevalent solanaceous vegetable fruit crop in the world and also in India. It is herbaceous in nature containing monocarpic and self-pollinated flowers but cross-pollination also occurs in a certain percentage. It is also a tropical and day neutral plant, tomatoes nutritional value is high which contained in a high amount of vitamin A and C, fibers, minerals, organic acid and both essential and non essential amino acids. Tomato also contains Beta-carotene and lycopene pigments, phosphorus, and iron, which play a vital role in Indian diets.

*Ralstonia solanacearum* is one of the most destructive pathogens in the world. It has a wide range of host crops across 54 families and more than 450 species of crops (Wicker *et al.*, 2007). A study revealed that in the summer season 10 to 100% incidence of vegetable bacterial wilt occurs (Kishun, 1985)<sup>[4]</sup>, Singh *et al.* (2010)<sup>[12]</sup> reported that 2 to 95% bacterial wilt incidence occurred in tomato. *Ralstonia solanacearum* invades xylem vessels to cause wilt in tomato, pathogen persists in soil for several months to years and infests plant parts when the favourable condition occurs (Genin and Denny, 2012)<sup>[3]</sup>, the ability of pathogenicity is dependent upon its number or density, after reaching of its appropriate density it activates certain pathogenicity genes, which does not happen on low densities (Schell, 2000). It is very complicated to control *Ralstonia solanacearum* because of its complex mechanism of pathogenicity, endophytically growing nature, persistence in soil, dispersal through water and association with certain weeds (Wang and Lin, 2005). Symptoms of bacterial wilt of tomato caused by *Ralstonia solanacearum* characterized by loss of turgidity followed by drying of leaves and then dropping, sudden wilting also occurs in case of bacterial wilting. The plant does not recover at night or even after irrigation. The present study was conducted to evaluate the efficacy of certain antibacterial chemicals under *in vitro* conditions against *Ralstonia solanacearum*.

### Review of Literature

Ritu *et al.* (2021) evaluated six antibacterial chemicals against *R. solanacearum* causing potato bacterial wilt and reported that chloramphenicol showed the highest antibacterial activity at 100 ppm and 250 ppm with 15.96 and 19.60 mm inhibition zone respectively, whereas streptomycin at 500 ppm showed highest inhibition zone with 28.03 mm. Akash *et al.* (2021)<sup>[1]</sup> evaluated the efficacy of eight chemicals and their combinations against *R. solanacearum* in

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which streptocycline (500 ppm) + bleaching powder (3000 ppm) + copper oxychloride (3000 ppm) showed the highest inhibition zone with 26.66 mm, the least inhibition zone was formed in bleaching powder alone with a mean inhibition zone of 10.33 mm. Laiphrakpam *et al.* (2020) [6] found that streptocycline (100 ppm), tetracycline (200 ppm) and ciprofloxacin (300 ppm) showed the highest inhibition zone with 25.6 mm, 27.2 mm and 33.4 mm respectively among the different chemicals. Nagaraja *et al.* (2018) [8] conducted an experiment as an inhibition zone assay method to evaluate the efficiency of certain antibacterial chemical and their combination, streptocycline + copper oxychloride at 500 ppm + 3000 ppm produce the highest inhibition zone with 29.38 mm, whereas at 500 ppm of streptocycline produce 27.07 mm inhibition zone, followed by all other chemicals *viz.*, kasugamycin, plantomycin, copper oxychloride and copper hydroxide showed the effective result. Bawari *et al.* (2019) [2] evaluated that amoxicillin, streptocycline, cefixime, tetracycline, ciprofloxacin and norfloxacin showed the highest inhibition zone at 500 – 750 ppm. The highest inhibition zone recorded in amoxicillin at 500 to 750 ppm was 31.25 and 36.00 respectively, followed by cefixime 31.25 to 35.50 mm, ciprofloxacin 28.75 to 32.75 mm, tetracycline 24.25 to 27.25 mm, norfloxacin 20.75 to 22.75 mm, and streptocycline 14.75 to 16.25 mm at 500 and 750 ppm respectively. Similarly, streptocycline and 2 Bromo – 2 Nitropropane – 1, 3 – diol antibiotics were found to check the *Ralstonia solanacearum* by several workers (Premchand *et al.*, 2016; Revathi *et al.*, 2017; Roop *et al.*, 2017; Raghu, 2011; Tao *et al.*, 2011 and Mohsin *et al.*, 2016) [9, 11, 10, 14, 7].

### Materials and Methods

There are three antibiotics streptocycline, 2 Bromo – 2 Nitropropane – 1, 3 – diol (bacterinash) and validamycin, one fungicide Copper oxychloride and one plant derived antibacterial chemical bacterisan composed by clove extract 1%, black pepper extract 0.3%, basil extract 0.3%, protein 5%, silicon 0.5% and adjuvants 92.9% were evaluated for to know their best efficacy against *R. solanacearum* under *in vitro* conditions at different concentrations by inhibition zone assay method. In this process all the glassware were sterile in hot air oven at 180 °C at 20 minutes, CPG media were formed by adding Casamino acid (casein hydrolysate) 1g, Peptone 10g, Glucose 5g and Agar 17g at per liter of sterile water, and then autoclave at 121°C at 30 minutes, after autoclave cooled the media and mix 0.6g antibiotics and 5ml of 1% stock solution per lit of CPG media then formed media were poured in 150 mm sterile petriplates, then allow to solidify. All the test antibacterial chemicals were prepared separately at different concentrations. 5 mm diameter of Whatman No.42 (filter paper disc) were soaked in their respective antibacterial chemical solutions for 15 to 20 minutes. Test bacterium of  $2 \times 10^8$  cfu/ml were added to sterile water and then poured with 25 µl in prepared CPG plates and then evenly spread by spreader, after this process inoculated plates were kept in

refrigerator for 15 to 20 minutes. Filter paper disc treated with their respective antibacterial chemicals were placed in their respective place in prepared petriplates treated with test bacterium and then incubated for 48 hours at 28°C temperature. Observations were recorded by forming inhibition zone around the filter paper disc at 24 to 96 hours of inoculation.

### Result and Discussion

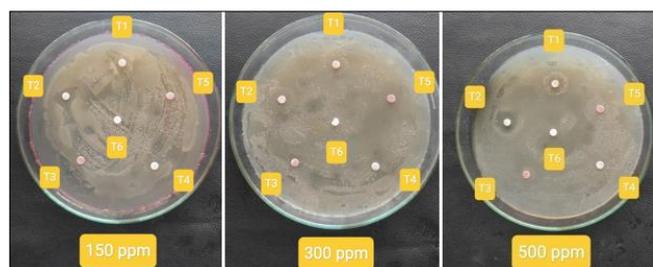
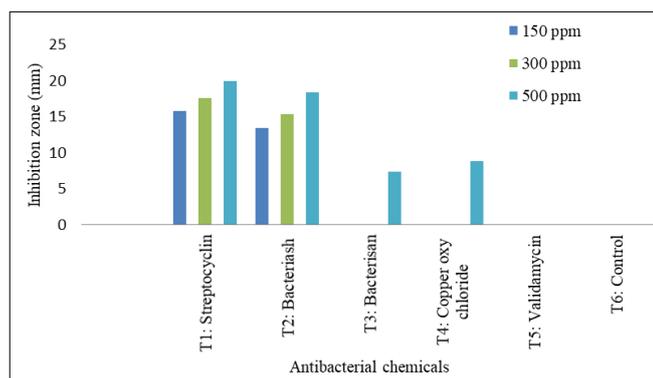
Three antibiotics (streptocycline, 2 Bromo – 2 Nitropropane – 1, 3 – diol and validamycin), one fungicide (Copper oxychloride) and one plant derived antibacterial chemical (bacterisan) were evaluated by inhibition zone assay method for their efficacy against *R. solanacearum* under *in vitro* condition. The results revealed in table 1 that the streptocycline showed highest inhibition zone of 20.00 mm at 500 ppm concentration among the all antibiotics tested followed by 2 Bromo – 2 Nitropropane – 1, 3 – diol showed significant effect to produce 18.40 mm inhibition zone at 500 ppm concentrations. After that streptocycline 300 and 150 ppm showed 17.60 and 15.80 mm inhibition zone respectively. 2 Bromo – 2 Nitropropane – 1, 3 – diol showed moderate effect to produce 15.40 and 13.40 mm inhibition zone at 300 and 150 ppm concentration respectively. Whereas copper oxychloride showed 8.80 mm inhibition zone at 500 ppm, while bacterisan showed least effect with 7.40 mm at 500 ppm concentration, bacterisan and copper oxychloride dose not show any inhibition zone at 150 and 300 ppm concentration. However, validamycin were found to be non significant result at all concentrations against *R. solanacearum*.

In the present investigation, streptocycline at 500 ppm showed highest inhibition zone with 20.00 mm, followed by 2 Bromo – 2 Nitropropane – 1, 3 – diol showed inhibition zone 18.40 mm at 500 ppm. Whereas copper oxychloride and bacterisan formed inhibition zone 8.80 and 7.40 mm respectively at 500 ppm only. The findings of the present study are supported by Singh and Jagtap (2017) [13] they also found highest inhibition zone with 18.4 mm and 21.7 mm in the use of streptocycline at 400 and 500 ppm respectively followed by copper oxychloride showed 10.6 and 11.6 mm inhibition zone at 1500 and 2000 ppm respectively. The present results also supported by Nagaraja *et al.* (2018) [8], they found streptocycline superior combine with copper oxy chloride and receive highest inhibition zone with 27.07 mm at 500 ppm. Similar trends was also recorded by Laiphrakpam *et al.* (2020) [6], where streptocycline showed highest inhibition zone with 25.6 mm in diameter followed by ciprofloxacin 25.4 mm and bacterinashak 16 mm at 100 ppm concentration. Ritu *et al.* (2021) also observed that streptocycline 500 ppm showed highest inhibition zone with 28.03 mm at 500 ppm concentration while chloramphenicol showed significant effect to produce 23.96 mm inhibition zone at 500 ppm whereas, streptomycin showed least effect to produce 9.30 mm inhibition zone at 500 ppm concentration.

**Table 1:** *In vitro* evaluation of antibiotics against *Ralstonia solanacearum*

Tr. No.	Treatments	Mean inhibition zone (mm)		
		150ppm	300ppm	500ppm
T1	Streptocyclin	15.80 (04.09)	17.60 (04.31)	20.00 (04.58)
T2	2 Bromo – 2 Nitropropane – 1, 3 - diol	13.40 (03.79)	15.40 (04.04)	18.40 (04.40)
T3	Bacterisan	00.00 (01.00)	00.00 (01.00)	07.40 (02.89)
T4	Copper oxychloride	00.00 (01.00)	00.00 (01.00)	08.80 (03.11)
T5	Validamycin	00.00 (01.00)	00.00 (01.00)	00.00 (01.00)
T6	Control	00.00 (01.00)	00.00 (01.00)	00.00 (01.00)
	S.E.(m)±	0.017	0.017	0.067
	C.D. @ (1%)	0.049	0.05	0.197
	C.V.(%)	1.866	1.838	5.283

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

**Fig 1:** *In vitro* evaluation of antibiotics against *Ralstonia solanacearum***Fig 2:** *In vitro* evaluation of antibiotics against *Ralstonia solanacearum*

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