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

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Abstract

Ralstonia solanacearum is a devastating pathogen that causes bacterial wilt in chilli plants, leading to significant crop losses. Effective management strategies are crucial to combat this disease. In this study, we conducted an *in vitro* evaluation of antibiotics against *Ralstonia solanacearum* using HiMedia Octa and Hexa Discs, with a focus on determining the most effective antibiotics and concentrations for inhibiting the pathogen's growth. The results revealed that Ofloxacin exhibited the highest inhibitory effect against *Ralstonia solanacearum*, forming a maximum inhibition zone of 3.7 mm at a concentration of 5 mcg. Co-Trimoxazole also demonstrated significant inhibition, with a maximum zone of 3.5 mm observed at a concentration of 25 mcg. These findings suggest that Ofloxacin and Co-Trimoxazole have strong potential for controlling *Ralstonia solanacearum* in chilli plants. Furthermore, the use of HiMedia Octa and Hexa Discs provides a reliable method for assessing the antibiotic susceptibility of this pathogen.

Keywords: *Ralstonia solanacearum*, antibiotics, inhibition, chilli

Introduction

Ralstonia solanacearum, a Gram-negative bacterium, is a notorious pathogen responsible for causing bacterial wilt, a devastating disease in chilli (*Capsicum annuum* L.) plants worldwide. This pathogen exhibits a remarkable ability to survive in soil and water, making it a persistent threat to chilli cultivation. Bacterial wilt leads to severe economic losses in chilli production, affecting both smallholder farmers and the agricultural industry at large. *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) [8]. Managing this disease is challenging due to its ability to persist in the soil for extended periods and its wide host range, which includes many solanaceous crops. Antibiotics have been explored as a potential means of controlling *Ralstonia solanacearum*. Understanding the susceptibility of this pathogen to different antibiotics and their optimal concentrations is essential for developing effective management strategies. *In vitro* evaluation of antibiotics offers a controlled environment to assess the inhibitory potential of various antimicrobial agents. In this study, we investigate the *in vitro* effectiveness of selected antibiotics against *Ralstonia solanacearum* in chilli plants, employing HiMedia Octa and Hexa Discs as a standardized testing platform. HiMedia Octa and Hexa Discs provide a convenient and reliable method for assessing the susceptibility of *Ralstonia solanacearum* to these antibiotics, offering valuable insights into the development of effective disease management strategies in chilli cultivation. The results of this study may hold promise for enhancing chilli crop resilience and reducing losses due to bacterial wilt.

Materials and Methods

In vitro evaluation of antibiotics

1. The bacterium culture of *Ralstonia solanacearum* was freshly inoculated in tubes containing autoclaved nutrient broth medium and incubated at 28±2 °C for 72 hours.
2. Around 200 µL bacterial suspension taken from tubes after growth was spread on plates containing nutrient agar with help of spreader and the HiMedia discs (Combi - 78, Universal - 1, Hexa G – minus 3 and Hexa Universal 4) were placed at appropriate positions.
3. The plates were incubated at 28±2 °C for 72 hours and observed for the production of inhibition zone around the discs.
The results were analysed statistically.

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Results and Discussion

Efficacy of distinct antibiotics at different concentrations. The 4 antibiotics HiMedia Hexa and Octa discs (Table 1-4) tested against isolate proved to be effective of all antibiotics,

Ofloxacin exhibited the highest inhibitory activity of 03.70 mm at 5 mcg concentration followed by Co-Trimoxazole exhibited inhibitory activity of 03.56 mm at 25 mcg concentration. (Bawari M. R. and T. Narendrappa 2019)^[3].

Table 1: Efficacy of Combo – 78 (HiMedia Octa Disc)

Treatment	Antibiotics	Symbol	Concentration	Zone of inhibition in 'mm'
T ₁	Ceftriaxone	CTR	30 mcg	00.00
T ₂	Gentamicin	GEN	10 mcg	00.00
T ₃	Co-Trimoxazole	COT	25 mcg	00.00
T ₄	Levofloxacin	LE	5 mcg	03.10
T ₅	Netillin	NET	30 mcg	01.50
T ₆	Tetracycline	TE	30 mcg	02.00
T ₇	Amoxyclav	AMC	30 mcg	00.00
T ₈	Ofloxacin	OF	5 mcg	03.00
T ₉	Control			00.00
SE ± (mean)				0.02
CD (P=0.01)				0.09

Table 2: Efficacy of Universal – 1 (HiMedia Octa Disc)

Treatment	Antibiotics	Symbol	Concentration	Zone of inhibition in 'mm'
T ₁	Gentamicin	GEN	10 mcg	01.60
T ₂	Amikacin	AK	30 mcg	01.20
T ₃	Ciprofloxacin	CIP	5 mcg	03.00
T ₄	Cefoxitin	CX	30 mcg	01.50
T ₅	Amoxyclav	AMC	30 mcg	00.00
T ₆	Tetracycline	TE	30 mcg	02.00
T ₇	Chloramphenicol	C	30 mcg	001.80
T ₈	Co-Trimoxazole	COT	25 mcg	00.00
T ₉	Control			00
SE ± (mean)				0.02
CD (P=0.01)				0.09

Table 3: Efficacy of Hexa G – minus 1 (HiMedia Hexa Disc)

Treatment	Antibiotics	Symbol	Concentration	Zone of inhibition in 'mm'
T ₁	Co-Trimoxazole	COT	25 mcg	03.50
T ₂	Amoxyclav	AMC	30 mcg	01.30
T ₃	Gentamicin	GEN	10 mcg	03.00
T ₄	Tetracycline	TE	30 mcg	02.00
T ₅	Ofloxacin	OF	5 mcg	03.70
T ₆	Cefuroxime	CXM	30 mcg	02.50
T ₇	Control			00.00
SE ± (mean)				0.01
CD (P=0.01)				0.05

Table 4: Efficacy of Hexa Universal 4 (HiMedia Hexa Disc)

Treatment	Antibiotics	Symbol	Concentration	Zone of inhibition in 'mm'
T ₁	Ampicillin	AMP	10 mcg	01.60
T ₂	Erythromycin	E	15 mcg	00.00
T ₃	Ampicillin/Sulbactam	A/S	10/10 mcg	02.00
T ₄	Gentamicin	HLG	120 mcg	03.00
T ₅	Penicillin – G	P	10 units	02.00
T ₆	Ceftriaxone	CTR	30 mcg	03.00
T ₇	Control			00.00
SE ± (mean)				0.01
CD (P=0.01)				0.05

Conclusion

The findings highlight the importance of antibiotic sensitivity testing in identifying suitable antibiotics for the management of bacterial infections, with Ofloxacin and Co-Trimoxazole showing promise as effective choices in combating the isolate.

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