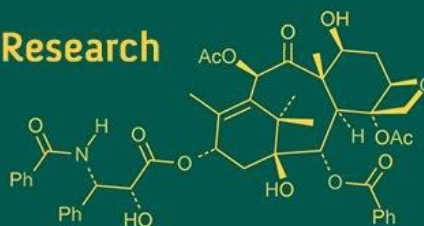


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; SP-8(10): 297-300
www.biochemjournal.com
Received: 08-06-2024
Accepted: 15-07-2024

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In-vitro evaluation of different bactericides and bioagents against *Ralstonia solanacearum*

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DOI: <https://doi.org/10.33545/26174693.2024.v8.i10Sd.2467>

Abstract

Ralstonia solanacearum is a well-known plant pathogen that causes bacterial wilt in tomato plants, leading to significant yield losses and economic challenges for growers. Addressing the impact of this pathogen necessitates the development of effective control strategies. This study investigates the *in vitro* efficacy of various bactericides against *Ralstonia solanacearum*, with a particular focus on their potential to manage bacterial wilt in tomatoes. A thorough screening of several bactericides was conducted, including Validamycin (3%), Streptomycin sulphate, Streptocycline, Copper oxychloride, Streptomycin sulphate + Streptocycline, Streptomycin sulphate + Copper oxychloride. These were tested at concentrations of 300 ppm using *in vitro* assays. The results revealed that Streptomycin sulphate in combination with Copper oxychloride, as well as Streptocycline alone, showed promising potential as effective strategies for controlling *Ralstonia solanacearum* in tomato plants. This research offers valuable insights into alternative methods for managing bacterial wilt, providing tomato growers with new avenues for disease control and improved crop protection.

Keywords: *Ralstonia solanacearum*, bactericides, bio-agents, tomato, *Trichoderma* spp

1. Introduction

Tomato (*Solanum lycopersicum* L.) are not only a staple in global cuisines but also hold significant economic value as a widely cultivated crop. Yet, their cultivation is often threatened by numerous pathogens, particularly the notorious *Ralstonia solanacearum*. This bacterial agent is responsible for bacterial wilt, a severe disease that can devastate yields and inflict financial strain on tomato producers. *Ralstonia solanacearum* presents a major challenge due to its extensive host range and its ability to persist in the soil, complicating management efforts once it infiltrates a field. The pathogen targets the vascular system of tomato plants, causing wilting, stunted growth, and ultimately leading to plant death. The economic ramifications of *Ralstonia solanacearum* extend beyond mere yield losses, encompassing heightened production costs related to disease management and control strategies. As the global demand for tomato continues to rise, the urgency for effective and sustainable solutions to combat this pathogen becomes even more pronounced. Conventional methods, such as crop rotation and soil fumigation, often have limitations and may not sufficiently safeguard against this resilient threat. To address these challenges, innovative strategies for managing bacterial wilt in tomato plants are essential. One promising approach is the investigation of bactericides chemical agents capable of inhibiting or eliminating *Ralstonia solanacearum*. Conducting *in vitro* studies is critical for evaluating the effectiveness of these bactericides, as they provide controlled settings for initial screenings, determine minimum inhibitory concentrations, and assess impacts on the pathogen's virulence. This research aims to advance the fight against bacterial wilt in tomato plants by thoroughly evaluating various bactericides *in vitro* for their effectiveness against *Ralstonia solanacearum*. The outcomes of this study could empower tomato growers with practical tools for disease management, help lower production costs, and promote the stability of tomato production amid challenges posed by this resilient pathogen.

2. Materials and Methods

The present investigation on "*In-vitro* Evaluation of different bactericides and bioagents against *Ralstonia solanacearum*" was studied during 2023-2024 at Department of Plant

Pathology, College of Agriculture, Nagpur. The details of the material and methods used are as follows.

2.1 Isolation

Tomato plants showing symptoms of bacterial wilt, such as yellowing and wilting, were collected from affected fields. Infected root samples were cut into small pieces, disinfected with sodium hypochlorite, rinsed with distilled water, and air-dried. These pieces were then placed on sterilized nutrient agar (NA) medium and incubated at 28 ± 2 °C to promote bacterial growth. Once growth was observed, cultures were purified. *Ralstonia solanacearum* was identified based on its morphological characteristics using a compound microscope and confirmed through additional biochemical tests.

2.2 In -vitro evaluation of bactericides

The *in vitro* assessment of bactericides and bioagents evaluated the susceptibility of bacterial isolates to these treatments. Sensitivity tests were conducted using the paper disc method to determine the efficacy of each treatment.

2.2.1 Procedure

1. The study involved preparing the recommended doses of bactericides in sterile nucleus-free water and storing them in vials.
2. Paper discs, punched out from autoclaved filter paper, were soaked in the bactericides for 2 hours.
3. The discs were then placed on nutrient agar plates inoculated with *Ralstonia solanacearum* culture, which had been incubated for 72 hours.
4. The plates were further incubated and observed for the production of inhibition zones around the paper discs.
5. The results were analyzed statistically, with sterile distilled water-soaked paper discs serving as controls.

2.3 In -vitro evaluation of bioagents

2.3.1 Bacterial bioagents

1. The isolates of bacteria viz., *Bacillus subtilis* and *Pseudomonas fluorescens* were tested for their efficacy in inhibiting the growth of *Ralstonia solanacearum* by the paper disc method.
2. The bacterium culture of *Ralstonia solanacearum*, *Bacillus subtilis* and *Pseudomonas fluorescens* was freshly inoculated in tubes containing autoclaved nutrient broth medium and incubated at 28 ± 2 °C for 72 hours.
3. Small paper discs punched out from autoclaved filter paper (Whatman no. 42) measuring 5mm in diameter

were inserted in the respective vials containing bacterial culture of *Bacillus subtilis*, and *Pseudomonas fluorescens* and allowed to soak for 2 hours.

4. Around 200 µL bacterial suspension of *Ralstonia solanacearum* taken from tubes after growth was spread on plates containing nutrient agar with help of spreader and the paper discs were placed at appropriate positions.
5. The plates were incubated at 28 ± 2 °C for 72 hours and observed for the production of inhibition zone around the filter paper discs. The results obtained were analysed statistically. The paper disc soaked in sterile distilled water served as control.

2.3.2 Fungal bioagents

1. The fungal isolates were tested for their inhibitory effect on *Ralstonia solanacearum* *in vitro* by inhibition zone assay method.
2. All the fungal isolates were grown separately on Potato Dextrose Agar.
3. 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.
4. Around 200 µL bacterial suspension taken from tubes after growth was spread on plates containing nutrient agar with help of spreader.
5. Fungal discs of 5mm diameters from margin of actively growing four days old culture removed and placed in the center of agar plates.
6. The plates were incubated at 28 ± 2 °C for 4 days.
7. The observation on the zone of inhibition around the mycelial disc against *Ralstonia solanacearum* was recorded after the incubation period.

3. Results and Discussion

This study focused on tomato wilt caused by *Ralstonia solanacearum*. It involved several key steps: collecting samples, isolating the pathogen, identifying the isolated strain, and conducting pathogenicity tests to fulfill Koch's postulates. Additionally, the research evaluated the efficacy of bactericides and bioagents against *Ralstonia solanacearum* *in vitro*. The findings from these experiments are presented below.

3.1. In -vitro evaluation of bactericides

Efficacy of 6 bactericides at 300 ppm concentration was tested *in-vitro* by following the paper disc diffusion method against *Ralstonia solanacearum*.

Table 1: Efficacy of bactericides at 300 ppm concentrations on 24th, 48th and 72th hrs

Treatment	Bactericides	Concentrations 300 ppm		
		24 th hrs	48 th hrs	72 th hrs
	Zone of inhibition in ‘mm’			
T ₁	Validamycin 3%	8.20	8.87	9.97
T ₂	Streptomycin sulphate	12.33	14.63	15.43
T ₃	Streptocycline	14.97	18.80	20.43
T ₄	Copper oxychloride	9.63	10.30	11.87
T ₅	Streptomycin sulphate + Streptocycline	13.33	14.63	16.73
T ₆	Streptomycin sulphate + Copper oxychloride	13.93	15.13	17.37
T ₇	Absolute Control	00	00	00
F test		Sig	Sig	Sig
SE ± (mean)		0.29	0.28	0.23
CD (P= 0.01)		1.26	1.20	0.98

The data presented in Table. 1 revealed that Among the six bactericides tested against *Ralstonia solanacearum*, Streptocycline demonstrated the highest inhibitory activity, with a zone of inhibition of 20.43 mm at a concentration of 300 ppm. This was followed by Streptomycin sulphate + Copper oxychloride and Streptomycin sulphate + Streptocycline, which exhibited zones of inhibition of 17.37 and 16.73 respectively at concentrations of 300 ppm and In contrast, Validamycin showed the least efficacy, with a zone of inhibition of only 9.97 mm at a concentration of 300 ppm.

In a similar way Singh and Jagtap (2017) ^[15] studied *In vitro* the efficacy of antibacterial chemicals against. Bawari (2014) ^[3]. He reported that Streptocycline was highly effective with the maximum inhibition zone of 31.25 to 36.00 mm at 500 to 750 ppm. Gupta and Razdan (2013) ^[6] evaluated *in vitro* eleven antibiotics against *R.solanacearum*, causing brinjal wilt and reported that antibiotic plantomycin (streptomycin sulphate + tetracycline) at 100, 250 and 500ppm gave highest inhibition zone of 10.83,18.63 and 28.77 mm, respectively.

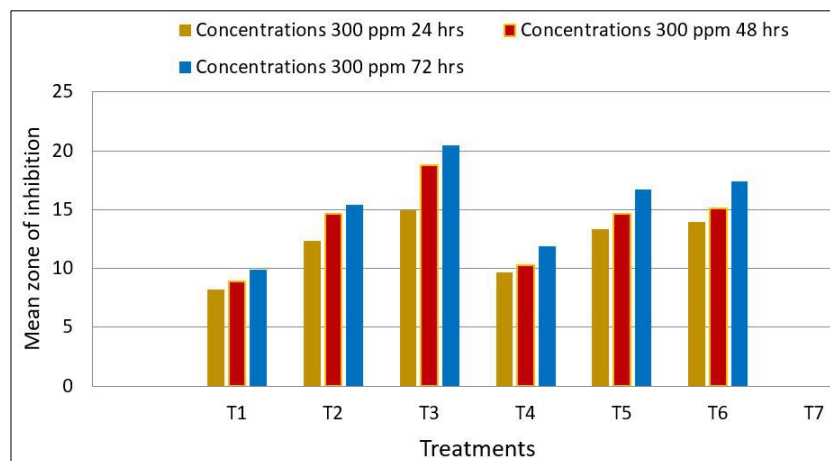


Fig 1: Efficacy of bactericides at 300 ppm concentrations on 24th, 48th and 72th

3.3 *In -vitro* evaluation of bio-agents against *Ralstonia solanacearum*

The effectiveness of various bioagents against *Ralstonia solanacearum* was evaluated using the paper disc diffusion method, and the results are presented in the following tables. These results demonstrate significant variations in the efficacy of different bioagents, indicating that some are more effective than others in controlling this bacterial isolate.

Among the four bioagents tested against *Ralstonia solanacearum* (Table 2), *Pseudomonas fluorescens* demonstrated the highest inhibitory activity, with a zone of inhibition of 13.75 mm. *Bacillus subtilis* also showed significant activity, with a zone of inhibition of 10.63 mm. In contrast, the fungal bioagents tested were ineffective, failing to produce any inhibition zones and instead being inhibited by the bacterial isolate itself. These findings are in consonance with reports of several earlier workers. Singh,

R. and Jagtap, G. P., (2017) ^[15] bioagents against *Ralstonia solanacearum* infecting bacterial wilt in ginger. Khan *et al.*, (2018) ^[8] evaluated the three potential microbial bioagents, viz. *Trichoderma harzianum*, *Bacillus thuringiensis*, *Pseudomonas fluorescens* against bacterial wilt of lettuce under hydroponic culture system.

Table 2: *In -vitro* evaluation of bio-agents against *Ralstonia solanacearum* on 48 hrs

Treatment	Bioagents	Zone of inhibition in 'mm'
T ₁	<i>Bacillus subtilis</i>	10.63
T ₂	<i>Pseudomonas fluorescens</i>	13.75
T ₃	<i>Trichoderma viride</i>	00
T ₄	<i>Trichoderma harzianum</i>	00
T ₅	Control	00
F test		Sig
SE ± (mean)		0.23
CD (P=0.01)		0.99

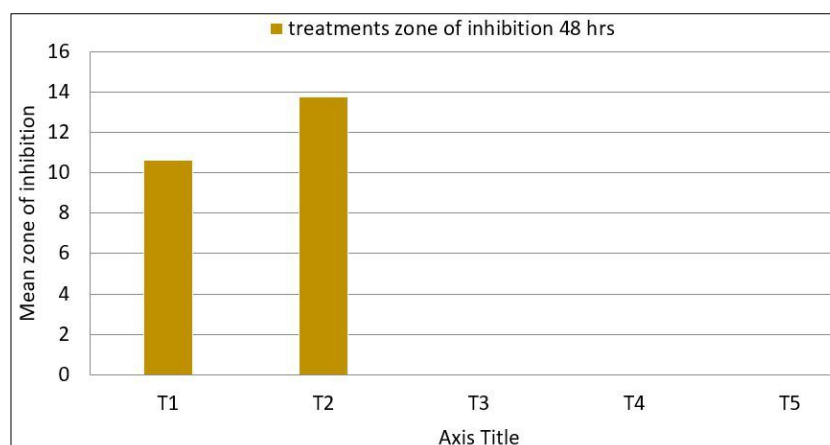


Fig 2: Efficacy of distinct bioagents on *Ralstonia solanacearum* on 48 hrs

4. Conclusion

The salient features of the present investigation are as under

1. *In vitro* evaluation of bactericides against *Ralstonia solanacearum* showed that Streptocycline had the highest inhibitory effect, with a 20.43 mm inhibition zone at 300 ppm.
2. *In vitro* evaluation of bioagents against *Ralstonia solanacearum* showed that *Pseudomonas fluorescens* had the highest inhibitory effect at 13.75 mm

5. Acknowledgement

I am thankful to the Head of the Plant Pathology section, College of Agriculture, Nagpur for providing all the necessary facilities for conducting the research work.

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