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

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Abstract
Ralstonia solanacearum is a devastating plant affecting chilli pepper plants worldwide. In this study, we investigated the inhibitory potential of various bioagents, including *Bacillus subtilis, Pseudomonas solanacearum, Bacillus megaterium, Trichoderma viride* and *Trichoderma harzianum*, against *Ralstonia solanacearum in vitro*. Our results revealed varying degrees of inhibition among these bioagents. Among the tested bioagents, *Bacillus megaterium* exhibited the highest inhibitory activity, with an impressive 01.20 mm inhibition zone. In contrast, both fungal bioagents *Trichoderma viride* and *Trichoderma harzianum* showed no discernible inhibition (00.00 mm). These findings suggest that *Bacillus megaterium* holds promise as a potential biocontrol agent for managing *Ralstonia solanacearum* in chilli crop. Further research is warranted to explore the mechanisms underlying the inhibitory effects and the practical applications of these bioagents in disease management strategies.

Keywords: *Ralstonia solanacearum*, Bactericides, Inhibition, chilli

Introduction
Chilli pepper (*Capsicum* spp.) is an essential crop in the global agricultural landscape, providing a key ingredient in various cuisines and contributing significantly to the economies of many regions. However, the cultivation of chili peppers is frequently challenged by various pathogens, with *Ralstonia solanacearum* standing out as a formidable adversary. *Ralstonia solanacearum* is one the most destructive pathogen 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) [16]. Efforts to combat *Ralstonia solanacearum* have included a range of strategies, with a growing emphasis on the utilization of biocontrol agents. Plant pathogenic microbes have an immense impact on agricultural productivity, greatly reducing crop yield and sometimes causing total crop loss Antoun and Prevost (2006) [2]. Various fungi, actinomycetes and bacteria exhibited antagonistic effects against *R. solanacearum* (Kelman, 1953) [8]. Bioagents, such as *Bacillus subtilis, Pseudomonas solanacearum, Bacillus megaterium, Trichoderma viride,* and *Trichoderma harzianum*, have garnered attention for their potential to suppress the growth and spread of this devastating pathogen while minimizing the environmental impact associated with chemical interventions. In this study, we delve into the *in vitro* evaluation of these bioagents as potential tools for managing *Ralstonia solanacearum* in chili cultivation. Our investigation aims to assess the inhibitory capabilities of these bioagents and identify which, if any, exhibit the most promising results. Understanding the effectiveness of these biocontrol agents is a crucial step toward developing sustainable and environmentally friendly strategies for safeguarding chili pepper crops against bacterial wilt, ultimately ensuring a stable and productive chili pepper industry.

Materials and Methods
In vitro evaluation of bioagents
Bacterial bioagents
1. The isolates of bacteria *viz., Bacillus subtilis, Bacillus megaterium 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, Bacillus megaterium and Pseudomonas fluorescens* was freshly inoculated in tubes containing autoclaved nutrient broth medium and incubated at 28+2 ^{0}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, Bacillus megaterium 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 ºC for 72 hours and observed for the production of inhibition zone around the filter paper discs (Bawariand, M. R., T. Narendrappa, 2019) [4].
6. The results obtained were anaysed statistically. The paper disc soaked in sterile distilled water served as control.

Fungal bioagents
1. The fungal isolates were tested for their inhibitory effect on *Ralstonia solanacearum in vitro* by inhibition zone assay method.
2. *Trichoderma viride* and *Trichoderma harzianum* 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 ºC 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 5 mm 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 ºC for 4 days.
7. The observation on the zone of inhibition around the mycelial disc against *Ralstonia solanacearum* was recorded after the incubation period (Bawariand, M. R., T. Narendrappa, 2019) [4].

Results and Discussion

Table 1: Efficacy of bioagents on isolate on 48 hrs

Treatment	Bioagents	Zone of inhibition in 'mm'
	Bacterial bioagents	
T_1	*Bacillus subtilis*	01.10
T_2	*Pseudomonas fluorescens*	00.80
T_3	*Bacillus megaterium*	01.20
	Fungal bioagents	
T_4	*Trichoderma harzianum*	00.00
T_5	*Trichoderma viride*	00.00
T_6	Control	00.00
SE ± (mean)		0.03
CD (P=0.01)		0.12

The 5 bioagents (Table 1) tested against the isolate proved to be effective of all bioagents, *Bacillus megaterium* exhibited the highest inhibitory activity of 01.20 mm followed by *Bacillus subtilis* exhibited the inhibitory activity of 01.10 mm. The fungal bioagents found to be ineffective in inhibiting the growth of *Ralstonia solanacearum*, as it did not produced the inhibition zone and isolate inhibited the growth of fungal bioagents. Bawariand, M. R., T. Narendrappa, (2019) [4] has shown the similar results regarding fungal bioagents.

Conclusion
The purpose of the research was to evaluate efficacy of various bioagents against *Ralstonia solanacearum in-vitro*. Thus, bacterial and fungal bioagents evaluated and *Bacillus megaterium* proved to be more efficient by forming inhibition zone of 01.20 mm followed by *Bacillus subtilis* exhibited activity of 01.10 my. The fungal bioagents found to be ineffective in inhibiting the growth of *Ralstonia solanacearum*, as it did not produced the inhibition zone and isolate inhibited the growth of fungal bioagents.

References
1. Agrios GN. Plant pathology. Elsevier; c1997.
2. Antoun H, Prevost D. Ecology of plant growth promoting rhizobacteria. PGPR: Biocontrol and biofertilization; c2006. p. 1-38.
3. Baker KF, Cook RJ. Biological control of plant pathogens. WH Freeman and Company; c1974.
4. Bawariand MR, Narendrappa T. *In vitro* evaluation of bioagents and antibiotics against *Ralstonia solanacearum* causing brinjal wilt. Journal of Pharmacognosy and Phytochemistry. 2019;8(5):2038-2041.
5. Gomez KA, Gomez AA. Statistical procedures for agricultural research. John wiley & sons; c1984.
6. Hassan S, Inam-UI-Haq M, Farah Naz M, Tahir I, Ali Z. *In vitro* investigations on host specificity of *Ralstonia solanacearum* among solanaceous crops and its biological control in tomato. Pakistan Journal of Botany. 48(3):1279-1287.
7. Kerr JR. Bacterial inhibition of fungal growth and pathogenicity. Microbial ecology in health and disease. 1999;11(3):129-142.
8. Kelman A. The bacterial wilt caused by *Pseudomonas solanacearum.* Tech. Bull., 99: North Carolina Agril. Exp. Sta., North Carolina, U.S.A; c1953, 192.
9. Myint L, Ranamukhaarachchi SL. Development of biological control of *Ralstonia solanacearum* through antagonistic microbial populations. International Journal of agriculture and biology. 2006;8(5):657-660.
10. Mohammed AF, Oloyede AR, Odeseye AO. Biological control of bacterial wilt of tomato caused by *Ralstonia solanacearum* using *Pseudomonas* species isolated from the rhizosphere of tomato plants. Archives of Phytopathology and Plant Protection. 2020;53(1-2):1-16.
11. Narasimha MK, Srinivas C. *In vitro* screening of bio antagonistic agents and plant extracts to control bacterial wilt (*Ralstonia solanacearum*) of tomato (*Lycopersicon esculentum*). Journal of agricultural technology. 2012;8(3):999-1015.
12. Nion YA, Toyota K. Recent trends in control methods for bacterial wilt diseases caused by Ralstonia solanacearum. Microbes and environments. 2015;30(1):1-11.
13. Sharma JP, Kumar S. Management of *Ralstonia* wilt of tomato through microbes, plant extract and combination of cake and chemicals. Indian Phytopath. 2009;62(4):417-423.
14. Singh R, Jagtap GP. *In vitro* evaluation of antibacterial chemicals and bioagents against *Ralstonia solanacearum* infecting bacterial wilt in ginger. International Journal of Current Microbiology and Applied Sciences. 2017;6(5):2034-2045.
15. Thongwai N, Kunopakarn J. Growth inhibition of *Ralstonia solanacearum* PT1J by antagonistic bacteria isolated from soils in the Northern part of Thailand. Chiang Mai J Sci. 2007;34:345-354.
16. Wicker E, Grassart L, Coranson-Beaudu R, Mian D, Guilbaud C, Fegan M, *et al*. *Ralstonia solanacearum* strains from Martinique (French West Indies) exhibiting a new pathogenic potential. Applied and environmental microbiology. 2007;73(21):6790-801.