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

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Abstract
Chilli (*Capsicum annuum* L.) is a widely cultivated and economically important crop globally, susceptible to various pathogens, with *Ralstonia solanacearum* being a significant bacterial pathogen responsible for causing devastating losses. Chemical pesticides have traditionally been employed to combat this pathogen, but concerns over environmental safety and resistance development necessitate the exploration of alternative control strategies. This study focuses on the *in vitro* evaluation of botanical extracts as potential biocontrol agents against *Ralstonia solanacearum* in chilli. A diverse range of botanical extracts, derived from various plant species *viz.*, Neem, Marigold, Datura, Bael, Kanher and Tulsi, were prepared at concentrations 5%, 10% and 20% and tested for their inhibitory effects on *R. solanacearum* growth and pathogenicity. The experimental results revealed promising antimicrobial activity of botanical extracts of Marigold and Nerium at 20% concentration against *R. solanacearum*. These botanicals demonstrated significant inhibition of bacterial growth. The experimental results revealed promising antimicrobial activity of several botanical extracts against *R. solanacearum*. This research highlights the potential of botanical extracts as eco-friendly and sustainable alternatives to chemical pesticides for managing *R. solanacearum* infections in chilli crops.

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

Introduction
Chilli (*Capsicum annuum* L.) stands as one of the most widely cultivated and economically significant vegetable crops globally, contributing to the culinary diversity and flavor profiles of countless cuisines. However, the production of this fiery crop is often threatened by various pathogens, among which *Ralstonia solanacearum* holds a notorious reputation. This bacterial pathogen, responsible for causing bacterial wilt, poses a severe threat to chilli cultivation, leading to substantial yield losses and economic hardships for farmers worldwide. Traditionally, chemical pesticides have been employed as the primary means of combatting *R. solanacearum* in chilli crops. While effective in the short term, this approach has raised several concerns, including environmental contamination, negative impacts on non-target organisms, and the development of pesticide-resistant strains of the pathogen. Consequently, there is a growing urgency to seek sustainable and environmentally friendly alternatives to mitigate the devastating effects of *R. solanacearum* on chilli production. Various plants have been mentioned in Ayurveda, an ancient Indian Sanskrit literature, for their therapeutic advantages (Kaushik, 1988) [4]. Botanical extracts have emerged as a promising avenue for biological control in agriculture. The natural plant products derived from plant species have the capacity to control diseases caused by viruses, bacteria and fungal pathogens (Guleria and Tiku, 2009) [3]. Many plant species have developed natural defense mechanisms against pathogens over millennia, producing secondary metabolites with potent antimicrobial properties. These bioactive compounds have the potential to inhibit the growth and pathogenicity of various plant pathogens, including *R. solanacearum*, without the environmental risks associated with chemical pesticides. This study focuses on the *in vitro* evaluation of botanical extracts as a potential biocontrol strategy against *R. solanacearum* in chilli crops. By harnessing the power of nature's own defenses, we aim to explore and harness the antimicrobial potential of botanical extracts to safeguard chilli production. Through a systematic examination of various botanical extracts and their inhibitory effects on *R. solanacearum*, this research seeks to provide a foundation for the development of sustainable and environmentally friendly solutions for managing this destructive bacterial pathogen.

Ultimately, this work has the potential to revolutionize chilli crop protection practices, offering growers a more ecologically sound and economically viable approach to combatting *R. solanacearum* and ensuring the continued availability of this beloved spice in kitchens around the world.

Materials and Methods

In vitro evaluation of botanicals

Sensitivity of the isolate was tested by paper disc assay method.

1. Desired concentration of botanicals extracts *viz.*, Neem, Marigold, Datura, Bael, Kanher and Tulsi were freshly prepared in sterile nucleus free water.
2. Before preparation of extract, each botanical were dipped in 2% Sodium hypochlorite (NaOCl) for one minute.
3. The extracts were prepared by grinding 1 g of washed plant part of 2 ml distilled water with mixture.
4. The mixture was transferred in 1 ml centrifuge tubes and centrifuged for 10 minutes at 10,000 rpm.
5. The supernatant obtained by centrifuging was transferred to another vial.
6. Small paper discs punched out from autoclaved filter paper (Whatman no. 42) measuring 5mm in diameter were inserted in the respective vials containing supernatant of different plant extracts and allowed to soak for 2 hours.
7. 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.
8. Around 200 µL bacterial suspension 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.
9. The plates were incubated at 28+2 ºC for 72 hours and observed for the production of inhibition zone around the filter paper discs. (Khan, R. A. A., B. Ahmad, M. Ahmad, A. Ali, I. Naz and M. Fahim, 2019) [5]

The results were analysed statistically. The paper disc soaked in sterile distilled water served as control.

Results and Discussion

Table 1: Efficacy of bactericides at different concentrations on 48 hrs

Treatment	Botanicals	Concentrations		
		5%	10%	20%
	Zone of inhibition in 'mm'			
T_1	*Nerium oleander*	01.30	01.20	01.50
T_2	*Azadirachta indica*	01.30	01.20	01.10
T_3	*Aegle marmelos*	01.30	01.20	01.30
T_4	*Ocimum sanctum*	01.10	01.20	01.00
T_5	*Tagetes spp.*	01.30	01.10	01.50
T_6	*Datura stramonium*	01.00	01.10	01.30
T_7	Control	00.00	00.00	00.00
SE ± (mean)		0.02	0.04	0.03
CD (P=0.01)		0.10	0.20	0.10

The 6 botanicals (Table 1) tested against the isolate proved to be effective of all botanicals, Marigold and Nerium exhibited the highest inhibitory activity of 01.50 mm at 20% concentration. Contrastingly, Tulsi and Dature recorded the lowest zone of 01.00 mm at 20% and 5% concentration respectively. Similarly, Kumari, R., R. K. Ranjan and A. K. Mishra, 2020 [6] shown similar results.

Discussion

The results indicate that all botanicals possess inhibitory properties, further investigations may be necessary to optimize concentrations and explore potential synergistic effects. These findings underscore the potential of botanical extracts as eco-friendly alternatives for disease management in agriculture, with Marigold and Nerium standing out as promising options for further exploration and practical application.

Conclusion

The purpose of the research was to evaluate efficacy of various botanicals against *Ralstonia solanacearum in-vitro*. Thus various botanicals at different concentrations evaluated and Marigold and Nerium proved to be more effective at 20% concentration by forming inhibition zone of 01.50 mm as compared to others.

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