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Rhizobacteria as a Biocontrol Agent Against Soil Borne Fungal Pathogen *Macrophomina phaseolina*

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ABSTRACT: Rhizobacteria play a crucial role in the growth of plants, either directly or indirectly. Rhizobacteria promote plant growth through Nitrogen fixation, nutrient supply, phytohormone synthesis, and mineral solubilization, while also acting as biocontrol agents by inhibiting pathogen growth. Rhizobacteria are widely used as biocontrol agents for fungal pathogen control due to their multiple utility as antifungal, antinematode, and plant growth promoting agents. One of the organisms that promotes plant growth and acts as an antagonist against soil-borne diseases is rhizobacteria. The severity of root rot is reported maximum in pulse crops. Macrophomina root rot causes subsequent reduction in plant growth. Rhizobacteria is being used in present research as a biocontrol agent to reduce the growth of soil borne fungal pathogen *Macrophomina phaseolina*. At the Agriculture Research Station in Ummedganj, Kota, and the College of Agriculture in Kota, soil samples and root samplings of chickpea rhizospheric soil were randomly selected from a wide range of locations. Ten isolates in total were taken from various soil samples taken from various research regions both before and after flowering. On nutritional agar, rhizobacteria were isolated. The biochemical characterisation of rhizobacterial isolates led to their classification as PR 1 through PR 10. The severity of *Macrophomina* root rot in chickpea was significantly reduced in vitro. According to the research, there is a way to lessen *Macrophomina* root rot by using dual culture techniques by using rhizobacteria that are hostile to *M. phaseolina* characterisation.

Keywords: Rhizobacteria, biocontrol, *Macrophomina phaseolina.*

INTRODUCTION

The widespread application of pesticides to manage plant diseases has altered the ecosystem of the soil, tainted subterranean water, and led to the emergence of resistant cultivars. Rhizobacteria which were being used extensively as plant growth promoting bacteria are now used as biocontrol agents, which are ecofriendly and inexpensive (Khan *et al*., 2022). Rhizobacteria are used as antifungal, antinematode, and plant growth-promoting agents, these are frequently employed as biocontrol agents for the management of fungal pathogens (Daulagala, 2021). Rhizobacteria produces products that inhibits the growth of soil borne pathogens which provide additional advantage to the plant roots (Shaikh and Sayyed 2015). Biological management measures are very compatible with sustainable agriculture because antagonistic rhizosphere bacteria suppress the growth of pathogenic microorganisms without upsetting the ecological balance (Boro *et al*., 2022). Additionally, the severity of Macrophomina root rot in chickpea was significantly reduced in vitro. According to these research, there may be a way to lessen Macrophomina root rot by using dual culture techniques to nodulate rhizobium strains that are hostile to *M. phaseolina* characterization (Kumar *et al*., 2021). In more than 500 plant species, *Macrophomina phaseolina* produces seedling blight, root rot, stem rot, and pod rot in this at least 40 hosts have been identified from India alone (Ghosh *et al*., 2018). The fungus is extensively dispersed throughout tropical and subtropical regions of the earth. Biological control agents have been discovered to safeguard and manage root diseases with reduction in the application of chemical fungicides (Tariq *et al.,* 2020). Thus, investigations to see the impact of Rhizobacteria on Macrophomina were conducted. At the Agriculture Research Station in Ummedganj, Kota, and the College of Agriculture in Kota, soil samples and root samplings of chickpea rhizospheric soil were randomly selected from different locations. Ten isolates in total were taken from various soil samples taken from various research regions. On nutritional agar, rhizobacteria were isolated. The biochemical characterisation of rhizobacterial isolates led to their classification as PR 1 through PR 10.The collection of the soil samples from chickpea rhizospheres in the field gives information about the extent of diseases affecting the crop and quality of beans in different locations.

MATERIALS AND METHODS

A. Isolation of rhizobacteria from chickpea rhizosphere

Samplings of root and soil system was done from chickpea rhizosphere, before flowering and after

flowering stage. Different sites were demarcated in chickpea growing fields at Agricultural Research Station, Ummedganj-Kota and College of Agriculture, Kota. The plants were carefully uprooted, labelled, put in poly bags and brought to laboratory for isolation of rhizobacteria.

The soil adhering loosely to the roots was washed thoroughly under running tap water. Root samples were crushed in the mortar with the help of pastle and shaken with 100 ml sterilized distilled water for 10-20 minutes to obtain bacterial suspension. Different dilution of this bacterial suspension was obtained through serial dilution technique and was processed for soil samples collected from rhizosphere. Suitable dilution of both rhizoplane and rhizosphere solutions was then plated on appropriate culture medium (Aneja, 2002). The culture plates were then incubated in an incubator at appropriate temperature for 24-48 h and colony growth was observed.

100 ml of liquid nutrient agar medium was inoculated with 5.0 g of soil samples and incubated for 7 days at 30°C. From these enriched samples isolation was performed following serial dilution method. Well separated individual colonies with light yellow, yellow and white pigments were marked and detected by viewing under U.V. light. The individual colonies were picked up with sterilized loop and transferred on fresh nutrient agar medium. The plates were incubated at 28± 2°C for 24 h the single colonies developed were subsequently transferred in King's B medium slants and the pure cultures so obtained were stored in refrigerator at 4°C till further processing. Further the isolates were subjected to biochemical characterization to distinguish the isolates among themselves. The rhizobacterial isolates were designated as PR 1 to PR 10.

B. Dual culture test (plant assay)

Screening of predictable fluorescent and non-fluorescent rhizobacterial isolates for their antagonistic activity against soil borne fungal pathogen *Macrophomina phaseolina* was performed by dual culture method (Skidmore and Dickinson 1976).

First the bacterial isolates were streaked on respective media plates and was incubated at 28°C for 3-4 days. Loopful bacterial isolate was streaked on the potato dextrose agar at one end which was pre-inoculated with 5 days old, 5 mm mycelial disc of test pathogen on the opposite side. Control plate was maintained by placing only pathogen mycelial disc on the plate without bacteria.

The plates were incubated at 28±1°C for 5 days. Inhibition of fungal growth was assayed by measuring the radial growth of the fungus and per cent growth inhibition was calculated by using the formula suggested by Vincent (1947).

$$\text{P.G.I.} = \frac{C - T}{C}$$

Where, P.G.I = Per cent growth inhibition; C = Growth in control; T = Growth in treatment.

RESULTS AND DISCUSSION

Screening of isolated rhizobacterial species for their anti-pathogenic activity against soil borne fungal pathogens *viz., Macrophomina phaseolina* was performed by dual culture method. All isolates under the test were screened by their potentiality to check the mycelial growth of soil borne fungal pathogens *viz*., *M. phaseolina.* After 7 days of incubation, the mycelial growth was measured and the inhibition of mycelial growth due to presence of antagonistic rhizobacteria was recorded as per cent growth inhibition**.**

In the present investigation none of the rhizobacterial isolates proved their ability to rest mycelial growth of *Macrophomina phaseolina.* Although maximum per cent growth inhibition was recorded for PR-3 (16.44%) as compared to the control, which was certainly followed by PR 2 (14.66%), PR 4 & 9 (13.33%) and PR 7 (13.11%). Least per cent mycelial growth inhibition was shown by PR 8 (9.22%) which was followed by PR 6 (10.33%), PR 5 (11.11%), PR 1 and PR 10 (12.66%) (Table 1, Plate 1, Fig. 1 a-d). However in all cases, mycelial growth of pathogen overlapped the bacterial growth after 4-5 days of incubation.

Table 1: Antagonistic activity of native rhizobacterial isolates against *Macrophomina phaseolina.*

Name of isolate	Average mycelial growth* (cm)	Percent growth inhibition (%)
PR1	7.86	12.66
PR2	7.68	14.66
PR3	7.52	16.44
PR4	7.80	13.33
PR5	8.00	11.11
PR6	8.07	10.33
PR7	7.82	13.11
PR8	8.17	9.22
PR9	7.80	13.33
PR10	7.86	12.66
Control	9.00	0.00

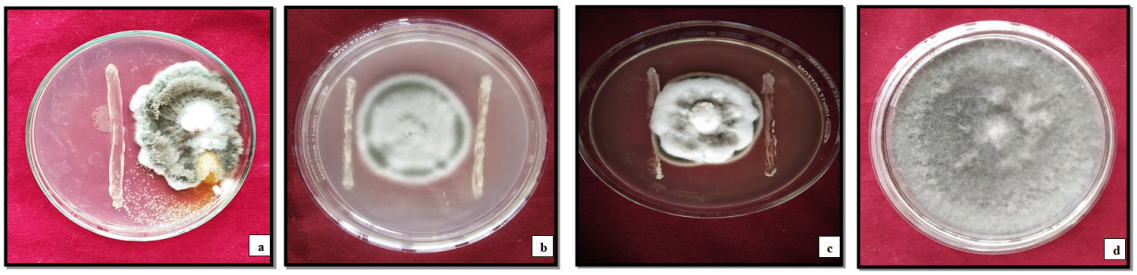

Plate 1. Evaluation of antagonistic activity of rhizobacterial isolates PR 3 against *Macrophomina phaseolina* causing root rot disease of chickpea (a, b & c) ; d- control.

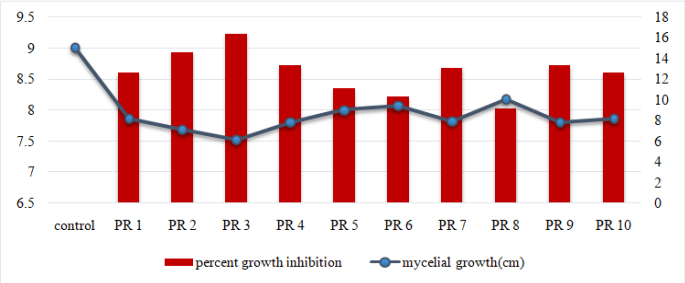

Fig. 1. Antagonistic activity of rhizobacterial (chickpea) isolates against *Macrophomina phaseolina* causing root rot disease of chickpea.

CONCLUSIONS

The rhizobacterial isolates showed different percent growth inhibition *i.e.*, PR 1 (12.66%), PR 2(14.66%), PR 3(16.44%), PR 4(13.33%), PR 5(11.11%), PR 6(10.33%), PR 7(13.11%), PR 8(9.22%), PR 9 (13.33%) and PR 10(12.66%). Although maximum growth inhibition was found with PR 3 (16.44%) while minimum growth inhibition was found with PR 8 (1.22%).

In conclusion, the study demonstrated that none of the rhizobacterial isolates were able to fully inhibit the growth of *Macrophomina phaseolina*. The isolates exhibited varying levels of growth inhibition, with PR 3 showing the highest inhibition at 16.44% and PR 8 showing the lowest at 9.22%. Despite these variations, all isolates had limited effectiveness in controlling the pathogen. These findings suggest that while certain rhizobacterial strains have some potential for managing *Macrophomina phaseolina*, their overall impact is modest and further research is needed to enhance their efficacy or explore alternative biocontrol strategies.

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