***Original Research Article***

***In vitro* evaluation of plant growth promoting rhizobacteria isolates against *Fusarium oxysporum* f. sp. *cicero***

# ABSTRACT

 Chickpea (*Cicer arietinum* L.) is one of the most important pulse crops, contributing significantly to global pulse production. However, its productivity is severely constrained by Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *ciceri*. The present study evaluates the antagonistic potential of native Plant Growth Promoting Rhizobacteria (PGPR) isolates collected from the Marathwada region, Maharashtra, against this pathogen. A total of 19 PGPR isolates were screened using an in vitro dual culture assay, where inhibition of fungal mycelial growth ranged from 39.63% to 86.67%. Among these, the isolate CPA2 exhibited the highest inhibition (86.67%), followed by CPP4 (85.22%) and CPJ1 (83.48%). The antagonistic activity of PGPR is attributed to the production of bioactive compounds such as siderophores, antibiotics, and hydrolytic enzymes that suppress pathogen growth. The study suggests that these native PGPR isolates have the potential to be developed as biocontrol agents for sustainable management of Fusarium wilt in chickpea cultivation.

**Keywords:** Chickpea, Wilt, *Fusarium oxysporum* f. sp. *ciceri*, PGPR, biocontrol, Isolates

## Introduction

 Chickpea (*Cicer arietinum* L.), commonly known as Bengal gram or garbanzo bean, is one of the world’s most important pulse crops ranking just after beans and peas. It plays a vital role in food and nutritional security, especially in countries like India, which alone contributes over 70% of global chickpea production. Rich in protein, fiber, vitamins, and essential minerals, chickpea is a key dietary staple and contributes to soil health through nitrogen fixation. According to the FAOSTAT 2023 report, globally, Bengal gram (chickpea) was produced on an area of approximately 149.5 lakh hectares, with a production of 165.2 lakh tonnes and a productivity of 1,104 kg/ha (FAO, 2023). India continues to be the largest producer, accounting for about 74.6% of global production. In the 2022–23 season, chickpea was cultivated over 110.91 lakh hectares in India, yielding 135.0 lakh tonnes with a productivity of 1,217 kg/ha (ICAR-IIPR, 2023). The output of all pulses in India during this period was dominated by chickpeas, contributing nearly 50% of the total pulse production. India is followed by Australia, Myanmar, and Ethiopia in global chickpea production (FAO, 2023).

In Maharashtra, during 2022–23, chickpea was cultivated over 10.12 lakh hectares, producing 18.73 lakh tonnes, with an average productivity of 1,851 kg/ha (Government of Maharashtra, 2023). Within the Marathwada region, the area under chickpea cultivation was 4.89 lakh hectares, with a production of 6.68 lakh tonnes and a productivity of 1,365 kg/ha, showing a significant increase compared to earlier years (Government of Maharashtra, 2023). Chickpea remains one of the major rabi pulse crops of this region.

Despite its importance, chickpea productivity remains low in many regions due to a range of biotic stresses chief among them is *Fusarium* wilt, caused by the soil-borne fungus *Fusarium oxysporum* f. sp. *ciceri* (FOC). This pathogen is particularly devastating, capable of wiping out entire crops under favorable conditions. In areas like Maharashtra’s Marathwada region, disease incidence has been reported at alarming rates. Once in the soil, the fungus can survive for years, making it extremely difficult to manage using traditional methods like crop rotation or chemical fungicides.

Chemical control methods, while effective to some extent, come with serious downsides soil health deterioration, resistance buildup in pathogens, harm to beneficial microbes, and concerns over food safety. As a result, there's growing interest in safer, sustainable alternatives for disease management.

One such promising solution lies in the use of **plant growth promoting rhizobacteria (PGPR)** naturally occurring soil bacteria that not only help plants grow better but also protect them from harmful pathogens. These beneficial microbes colonize the root zone (rhizosphere) and offer a range of advantages: they can boost nutrient uptake, produce plant hormones, and even act as natural defenders by producing antifungal compounds, enzymes, and siderophores. Some PGPR species are known to effectively suppress *Fusarium* wilt and can enhance the plant’s own defense systems, offering a dual benefit of growth promotion and disease control.

Given their potential, this study focuses on evaluating the ability of certain PGPR isolates to inhibit *Fusarium oxysporum* f. sp. *ciceri* under laboratory (*in vitro*) conditions. The goal is to identify promising microbial strains that could be used as eco-friendly, biological alternatives to chemical fungicides in chickpea cultivation.

**MATERIALS AND METHODS**

## 3.4 Collection of rhizosphere soil samples for PGPR isolates.

The rhizosphere soil samples were collected from healthy chickpea plants from three agroclimatic zones of the Marathwada region viz. Western Scarcity Zone, Central Plateau Zone, Eastern Transition Zone of Maharashtra, India. Soil samples were collected randomly at a depth of 0-15 cm using standard protocols. From each location, five random samples were collected and pooled together to get a representative composite soil sample by quartering technique. About 100 g of soil from each location was properly labeled with the requisite information, sealed, and stored in the refrigerator till further studies.

## Isolation

The rhizosphere samples were collected from healthy chickpea plants using standard protocol. After drying and processing, ten grams of soil sample was suspended in 90 ml of sterile water and serial dilutions of the suspensions were made in sterile water blanks. One milliliter of 10-4 to 10-7 dilutions was plated on Nutrient agar medium (NAM). The plates were incubated for 4-5 days at 28 ± 2 °C and observed light yellow off-white-coloured colonies. Based on the descriptions of Chhabra and Sharma (2019), PGPR isolates were identified. The isolates were purified and maintained on Nutrient agar medium (NAM) plates and stored at 4 °C.

## Antagonism of PGPR isolates.

The PGPR isolates were tested against the wilt-causing fungus *Fusarium oxysporum* f. sp*. ciceri* by dual culture method (Karimi *et al*. (2012). In dual culture, 20 ml of sterilized and cooled nutrient agar was poured into sterile petri plates and allowed to solidify. Bacterial culture of 5 mm diameter cut from the margin of 5 days actively growing cultures of both test pathogen and antagonists were placed opposite to each other on PDA in Petri plates (90 mm). The Petri plates with a disc of the *Fusarium* alone served as the control. Each treatment was replicated three times. The inoculated petri plates were incubated at 28 ± 2 °C for 7 days.

## Experiment Details

Design : CRD (Completely Randomized Design)

Treatments (No. of PGPR) : Twenty

Replications : Three

Location : Department of Plant Pathology, COA, Parbhani

Observations on linear mycelial growth of the test pathogen and biocontrol agents were measured, and percent growth inhibition of the test pathogen was calculated by applying the formula given by Vincent (1947) as follows.

 C - T

 Per cent inhibition (I) =……………….. × 100

 C

Where,

C= Colony growth of Pathogen in control (mm)

T= Colony growth of Pathogen in dual culture plate (mm)

# RESULTS AND DISCUSSION

 The antagonistic potential of nineteen plant growth-promoting rhizobacterial (PGPR) isolates against *Fusarium oxysporum* f. sp. *ciceri*, the causative agent of chickpea wilt, was assessed using the dual culture technique. All tested isolates were found to significantly inhibit the mycelial growth of the pathogen, with the percent inhibition ranging widely from **39.63% to 86.67%**. These results clearly indicate that the selected PGPR isolates possess promising antifungal activity, although their effectiveness varied considerably.

 Out of the nineteen isolates evaluated, **eight isolates stood out for their strong antagonistic effects**, exhibiting more than 70% inhibition of fungal growth. These isolates not only suppressed fungal development effectively but also reduced the radial growth of *F. oxysporum* f. sp. *ciceri* to a considerable extent, ranging from **12.00 mm to 25.87 mm**, compared to the control.

 Among the most effective isolates, **CPA2 demonstrated the highest inhibitory activity**, achieving **86.67% inhibition** of fungal growth with a minimal radial spread of **12.00 mm**. This isolate was closely followed by **CPP4**, which showed **85.22% inhibition** and a radial growth of **13.30 mm**. Both isolates were statistically at par and emerged as the most promising biocontrol candidates. **CPJ1** also displayed strong antifungal activity, inhibiting fungal growth by **83.48%** with a radial growth of **14.87 mm**.

 Further, isolates **CPN2**, **CPB2**, and **CPB1** recorded inhibition percentages of **79.63%**, **79.26%**, and **77.85%**, respectively. These were accompanied by radial growth values of **18.33 mm**, **18.67 mm**, and **19.93 mm**, showing that these isolates also possess considerable antagonistic potential. Additionally, **CPL2** and **CPO2** achieved inhibition levels of **74.07%** and **71.26%**, with corresponding radial growths of **23.33 mm** and **25.87 mm**, respectively.

 The variability observed in the degree of fungal inhibition across the different PGPR isolates reflects their **distinct antagonistic capacities**, likely influenced by their unique metabolic profiles and modes of action. Some isolates may produce a broad range of **secondary metabolites** such as antibiotics, aldehydes, ketones, hydrogen cyanide, or organic acids, which interfere with fungal growth directly. Others might engage in **indirect mechanisms**, such as **siderophore production** that competes for iron, thereby depriving the pathogen of essential nutrients required for its proliferation.

 This diversity in performance not only highlights the potential of PGPR as **biological control agents** but also emphasizes the importance of identifying and utilizing the most potent strains for effective management of chickpea wilt. These findings lay a solid foundation for future investigations into the biochemical and molecular pathways involved in PGPR-pathogen interactions, and for the development of eco-friendly, sustainable strategies for chickpea disease management.

**Table 1: *In vitro* evaluation of Plant Growth Promoting Rhizobacteria isolates against most virulent *Fusarium oxysporum* f. sp. *ciceri***

|  |  |  |  |
| --- | --- | --- | --- |
| **Sr. no.** | **Isolate code** | \* **Colony growth (mm)** | \* **Inhibition (%)** |
| 1 | CPP1 | 45.20 | 49.78(44.87) |
| 2 | CPP2 | 40.33 | 55.19(47.98) |
| 3 | CPP3 | 51.20 | 43.11(41.04) |
| 4 | **CPP4** | 13.30 | 85.22(67.39) |
| 5 | CPN1 | 54.33 | 39.63(39.01) |
| 6 | **CPN2** | 18.33 | 79.63(63.17) |
| 7 | CPN3 | 51.67 | 42.59(40.74) |
| 8 | **CPJ1** | 14.87 | 83.48(66.02) |
| 9 | CPJ2 | 39.67 | 55.93(48.40) |
| 10 | CPA1 | 45.87 | 49.04(44.45) |
| 11 | **CPA2** | 12.00 | 86.67(68.58) |
| 12 | CPL1 | 48.87 | 45.70(42.54) |
| 13 | **CPL2** | 23.33 | 74.07(59.39) |
| 14 | CPO1 | 38.67 | 57.04(49.05) |
| 15 | **CPO2** | 25.87 | 71.26(57.58) |
| 16 | **CPB1** | 19.93 | 77.85(61.93) |
| 17 | **CPB2** | 18.67 | 79.26(62.91) |
| 18 | CPH1 | 53.43 | 40.63(39.60) |
| 19 | CPH2 | 46.87 | 47.93(43.81) |
| 20 | Control | 90.00 | - |
| **SE(m)±** | 0.81 | 0.55 |
| **C. D. (P=0.01)** | 2.32 | 1.59 |

\*Figures in the parenthesis are angular transformed value

**CPP4**

**CPA2**

**CSN2**

**CSN2**

**CSB2**

**CAB1**

**CSO2**

**CML2**

**Plate 1: *In vitro* efficacy of eight Plant Growth Promoting Rhizobacteria isolates against most virulent**

***Fusarium oxysporum* f. sp*. ciceri***

The results were consistent with reports of Kala *et al.* (2016), Wavare *et al*. (2017), and Thaware *et al*. (2017), who reported the highest mycelial growth inhibition of *Fusarium oxysporum* f. sp. *ciceri* (81.59 %) by *Pseudomonas fluorescens*. Similarly, brigido *et al*. (2019) studied *in vitro* hyphal inhibition of *F*. *oxysporum* f. sp. *ciceris* race 5 and suppression of *Fusarium* wilt. Nineteen *Bacillus, Paenibacillus*, *Pseudomonas,* and *Stenotrophomonas* spp. of the 23 bacterial isolates in the study significantly (P < 0.05) inhibited the *in vitro* hyphal growth of *F. oxysporum* f. sp. *ciceris* race 5 However, the extent of inhibition varied among isolates. Kapali *et al*. (2016) also reported the highest inhibition (82.25 %) by *Pseudomonas fluorescens*. Whereas Kadam *et al*. (2019) recorded a higher rate of inhibition (86.49%) Similar findings were also reported by Landa *et al*. (2001), Hesamedian *et al*. (2009), Kandoliya and Vakharia (2013), Chandar *et al.* (2016), Kapali *et al*. (2016), Zaim *et al*. (2018), Pandey *et al.* (2022) and Dewi *et al.* (2023).

**Conclusion**

This study clearly shows that native Plant Growth Promoting Rhizobacteria (PGPR) from the Marathwada region have strong potential to control *Fusarium oxysporum* f. sp. *ciceri*, a major cause of wilt in chickpea crops. Out of the 19 PGPR isolates tested, three stood out CPA2, CPP4, and CPJ1—showing the highest levels of fungal growth inhibition, with CPA2 leading at 86.67%. These beneficial microbes are believed to work by producing natural compounds like siderophores, enzymes, and antibiotics that help suppress harmful fungi.

Considering how important chickpea is as a staple pulse crop especially in India finding sustainable ways to manage diseases like Fusarium wilt is critical. Using these native PGPR strains offers a promising, eco-friendly alternative to chemical fungicides, and they are already well adapted to local conditions, making them even more suitable for farmers in the region.

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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