Original Research Article

BIOLOGICAL MANAGEMENT OF FUSARIUM LENTIL WILT THROUGH EXPLORING THE POTENTIAL OF PSEUDOMONAD ISOLATES

Abstract

Lentil (*Lens culinaris*), also known as "masoor," is a crucial pulse crop in the *Leguminoceae* family known for their high protein and nutrient content. Wilt incited by *Fusarium oxysporum* f.sp. *lentis* can result in significant yield losses for lentil farmers. Chemical management through fungicides can reduce crop losses but has negative effects on the environment and human health. To overcome this, use of plant growth-promoting rhizobacteria (PGPR) as a bio-inoculant is gaining attention as a sustainable approach to manage plant diseases. PGPR are soil bacteria that live in the rhizosphere of plants and promote growth. The use of PGPR-based agents has increased in agriculture in recent years. In present investigation, isolation and evaluation of novel pseudomonad isolates were performed from various soil sources for controlling lentil wilt caused by *Fusarium oxysporum* f.sp. *lentis* and assess their ability to inhibit the pathogen *in vitro*.

Keywords: Lentil (*Lens culinaris*), *Fusarium oxysporum* f.sp. *lentis*, Lentil wilt Plant growth-promoting rhizobacteria (PGPR)

Introduction

The legume crop is unique in world because of its high protein content (15-35%), fiber, minerals, carbohydrates, and nutrients (**Nadia *et al*., 2019**). In the family, Leguminosae (*Fabaceae*), lentils (*Lens culinaris* M.) are a deployed species (2n =14) self-pollinating crop (**Dubey and Pandey, 2020**). Wilt of lentil is incited by *Fusarium oxysporum* Schlecht. emend. Snyder & Hansen f. sp. *lentis* Vasudeva and Srinivasan (Fol) (**Belabid and Fortas 2002)**. It brings about significant economic losses for lentil farmers in India and other nations depending on the severity and stage (pre-podding to pre-harvest) of wilt, yield loss could reach 0-100% (**Dubey, and Singh, 2018; Tiwari *et al*., 2018**). Pathogen can also survive within soil as chlamydospores, which can persist for several years (**Bayaa *et al*., 1997**). It has been shown that chemical-based management (fungicides) has the potential to reduce crop losses caused by plant-pathogenic organisms. However, due to the unfortunate and negligent application of synthetic pesticides, there can be

Comment [h1]: Biological management of lentil (*Lens culinaris* Medik) *Fusarium* wilt by using the potential *Pseudomonas* isolates

Comment [h2]: lentil (*Lens culinaris* Medik)

Comment [h3]: "massor",

Comment [h4]: A member of the *Leguminaceae* family known for their high protein and nutrient content.

Comment [h5]: Can result in significant losses upto ....%

Comment [h6]: Chemical management by fungicides reduces losses significantly but has some environmental drawbacks and conflicts on human health.

Comment [h7]: Instead other methods of control such as the use of plant ....which's gaining attention ....

Comment [h8]: In this study, isolation and evaluation of novel pseudomonas isolates were got from various soil sources and exploited for controlling lentil wilt caused by *Fusarium oxysporum* f.sp. *lentis* and assess their *in vitro* ability to inhibit the test pathogen.

Comment [h9]: Lentil, *Fusarium*, wilt & PGPR

Comment [h10]: The legume crops are unique due to their high protein content (27 – 34%) besides plenties of fiber, minerals, carbohydrates, and nutrients (**Nadia *et al*., 2019**).

Comment [h11]: Lentil (*Lens culinaris* M.) is a deployed species (2n =14) self-pollinating crop which belongs to the family Leguminosae (*Fabaceae*), (**Dubey and Pandey, 2020**). Wilt of lentil is incited by *Fusarium oxysporum* Schlecht. emend. Snyder & Hansen f. sp. *lentis* Vasudeva and Srinivasan (Fol) (**Belabid and Fortas 2002**).

Comment [h12]: It causes significant economic losses for farmers in India and other countries depending on the severity and crop stage (pre-podding to pre-harvest), yield loss could reach 100% (**Dubey, and Singh, 2018; Tiwari *et al*., 2018**). **Make sure that the mentioned percentage is in the cited manuscript? Anyhow, I didn't find it?**

Comment [h13]: **Bayaa *et al*., 1997 NOT FOUND IN THE REFERNCE LIST?!**

phytotoxicity and fungicidal residues that are harmful to the environment (natural enemies, flora and fauna) as well as human health (**Aioub *et al*., 2022; Eldeeb *et al*., 2022**). Use of plant growth-promoting rhizobacteria (PGPR) as a bio-inoculant is gaining attention as a sustainable approach to manage plant diseases and overcome the negative effects of fungicides. This is supported by recent research (**Backer *et al*., 2018; Ashok *et al*., 2015; Turan *et al*., 2021**). PGPR are soil bacteria that live in rhizosphere of plants and promote growth by various mechanisms. They can grow on, in, or around plant tissues (**Godbole *et al*., 2021**). For the past decade, use of PGPR-based agents (*Pseudomonas* spp.) as seed bio-inoculant, soil amendment, or soil drenching in crop production systems has increased in agriculture (**Abbouni *et al*., 2018**). Due to PGPRs potential to manage soil-borne pathogens by colonizing plant roots (**Hisamuddin *et al*., 2012**) and detoxifying the environment, they were considered a suitable solution for biological control (**Kumar *et al*., 2018; Abbouni *et al*., 2018; Adhikari *et al*., 2021**). The study aimed to isolate and evaluate PGPR from various soil sources for controlling lentil wilt. It was done under *in vitro* conditions and PGPRs were screened for their ability to inhibit pathogens and improve the environment.

Comment [h14]: ….and negligence in application ………… occurred in dismerits such as phytotoxicity, residues and risk to human and his environment as well

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Comment [h18]: Suitable biocontrol solutions are considered

Materials and Methods

Isolation and purification of pathogen

F. oxysporum was isolated and purified from wilt infected lentil plants. Samples were collected, surface sterilized, and transferred onto PDA medium (**Mondal *et al*., 2021**). Purification was done by growing hyphal tips and the pathogen was identified based on morphological characteristics. It confirmed Koch's postulate. The pathogen was maintained in refrigerated subcultures.

Rhizospheric soil samples collection and isolation of PGPR.

Rhizosphere soil was collected from different crops, and a bacterial suspension was obtained by shaking 1g of soil in sterilized water (**Godbole *et al*., 2021**). Bacterial isolation was done using KMB medium with benomyl and by dilution plate technique. Colonies of *Pseudomonas* spp. were purified and kept at 4°C. The isolates were evaluated for plant growth promotion and antagonistic potential (**Tsegaye *et al*., 2019**).

Evaluation of potential PGPR as antagonists against *F. oxysporum* for lentil wilt management

The potential of Pseudomonads spp. as antagonists against *Fusarium oxysporum* f.sp. *lentis* was evaluated in vitro by streaking bacterial isolates around pathogen in KMB medium and incubating at 28°C. Percent inhibition of mycelial growth was calculated using formula I = (C-T)/C x 100 (**Kalantari *et al*., 2018**).

Results and Discussion

Screening of native Pseudomonad isolates against *F. oxysporum* pathogen

Dual culture test revealed that all PGPRs (*Pseudomonad* isolates) have potential in controlling mycelium growth of *F. oxysporum* except isolate PGPR4 (**Fig.1 & Table 1)**. In this present study, out of 20 Pseudomonad isolates, PGP 18 exhibited highest percent mycelial growth inhibition (67.41%) followed by PGP 6, 16 and 17 (61.85, 61.48 and 59.63) respectively. Mycelial growth inhibition was 57.78%, 55.56% and 51.11% in isolates PGP 11, 15 and 20 respectively. Lowest percent of mycelial growth inhibition was exhibited by PGP 4 (0.00 %) followed by PGP 5, 1 and 2 (25.93, 29.26% and 30.37%) respectively as compared to control. Results of present study indicate that various PGPR pseudomonad isolates showed varying levels of inhibition against *F. oxysporum*, likely due to their varying antifungal abilities. The results of our study are partially or fully aligning with the findings those of Harsha *et al*. (2023), who evaluated 20 inherent *Pseudomonas* bacteria for their ability to combat *F. oxysporum* and found that isolate CRS-PF1 was particularly effective, exhibiting 51.84% inhibition of mycelium growth. The pseudomonad isolate PGPR-WS demonstrated strong inhibition against *F. oxysporum* in chickpeas, resulting in 75% reduction in growth of mycelia as compared to the control (**Kumar *et al*., 2018)**. Similarly, twenty-four strains of *Bacillus* (B4, B7 and B12) showed excellent antagonistic activity against investigated pathogenic fungi **(Abbouni, *et al*., 2018)**. Experimental findings were also in accordance with previous results (**Sachdev and Singh, 2018)** who identified and characterized *Pseudomonas aeruginosa* with positive antagonistic potentials toward *Fusarium lycopersici* and demonstrated radial inhibitions 67.85%. Isolation and antagonistic activity of rhizospheric bacteria against various disease-causing pathogens were also reported previously (**Sahu *et al*., 2017).**

Comment [h19]: Where, I = ..., C = ... and T = ...

Comment [h20]: %), respectively.

Comment [h21]: The lowest percent of mycelial growth inhibition was exhibited by PGP 4 (0.00 %) followed by PGP 5, 1 and 2 (25.93, 29.26% and 30.37%), respectively

Comment [h22]: this study are partially or fully go with the findings of Harsha *et al*. (2023), who evaluated the ability of 20 inherent *Pseudomonas* bacteria to combat *F. oxysporum* and found that isolate CRS-PF1 was particularly effective, exhibiting 51.84% inhibition of mycelium growth.

Comment [h23]: Which species? Is *B. thurengensis*?

Comment [h24]: A radial inhibition of 67.85%.

Table.1: Screening of native PGPRs (Pseudomonad isolates) against *F. oxysporum* f.sp. *lentis* (Fol) pathogen

Sl. No.	Pseudomonad isolates	Colony diameter *(mm)	Percentage inhibition of radial growth (PIRG)* (Fol)
1.	PGP1	63.67	29.26
2.	PGP2	62.67	30.37
3.	PGP3	60.67	32.59
4.	PGP4	90.00	0.00
5.	PGP5	66.67	25.93
6.	PGP6	34.33	61.85
7.	PGP7	52.00	42.22
8.	PGP8	48.33	46.30
9.	PGP9	48.00	46.67
10.	PGP10	46.00	48.89
11.	PGP11	38.00	57.78
12.	PGP12	50.67	43.70
13.	PGP13	46.00	48.89
14.	PGP14	46.00	48.89
15.	PGP15	40.00	55.56
16.	PGP16	34.67	61.48
17.	PGP17	36.33	59.63
18.	PGP18	29.33	67.41
19.	PGP19	47.67	47.04
20.	PGP20	44.00	51.11
	Control (KBs)	90.00	
C.D.		**3.84**	**3.32**
SEm (±)		**1.34**	**1.15**
C.V.		**4.68**	**4.26**

*Mean values of three replication

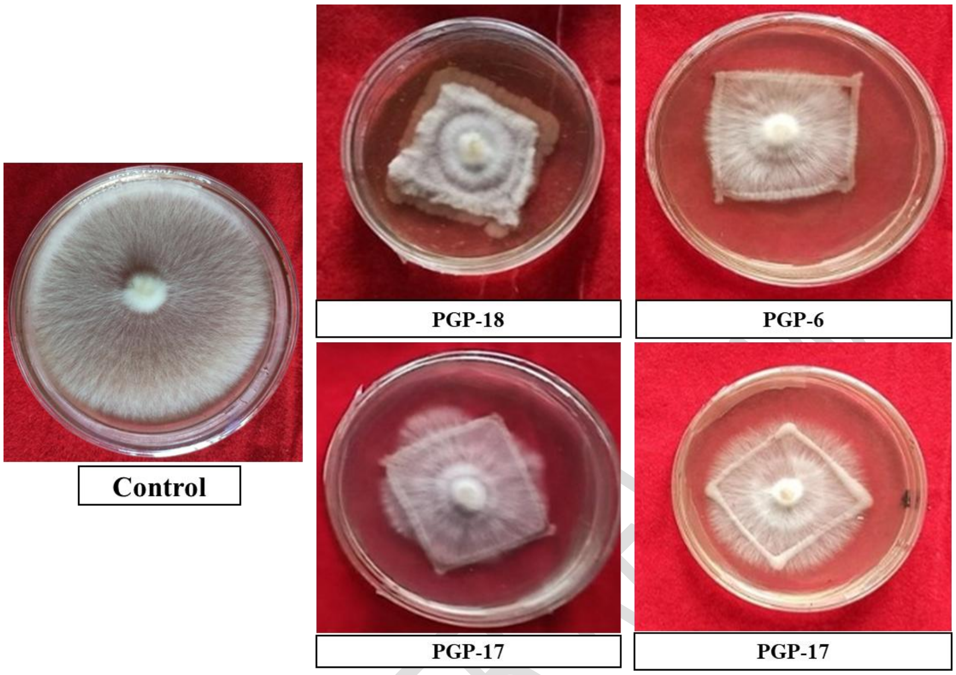

Fig. 1: Screening of native PGPRs (Pseudomonad isolates) against *F. oxysporum* f.sp. *lentis* (Fol) pathogen

Comment [h25]: NOT INFORMATIVE FOR THE FIGURE?!

Conclusion

The dual culture test demonstrated that most of the Pseudomonad isolates (PGPRs) were capable of inhibiting the growth of *F. oxysporum* f.sp. lentis (Fol), except for isolate PGPR4 (as shown in Fig.1 & Table 1). In this study, out of 20 PGPR isolates, PGP18 was found to be the most effective in inhibiting mycelial growth with 67.41% inhibition. PGP6, PGP16, and PGP17 also showed significant inhibition with 61.85%, 61.48%, and 59.63% respectively. PGP11, PGP15, and PGP20 showed moderate inhibition with 57.78%, 55.56%, and 51.11% respectively. Conversely, PGPR4 exhibited ~~the least~~ inhibition with 0.00%~~ compared to the control. The results suggest that the varying levels of inhibition displayed by the PGPR pseudomonads against *F. oxysporum* f.sp. lentis could be attributed to their differing antifungal abilities.~~

Comment [h26]: except for isolate PGPR4 (mention the percent inhibition & NOT the Table 1, this is a conclusion?!).

Comment [h27]: The PGP18 isolate was found the most effective, among 20 isolates, and produced 67.41% inhibition.

Comment [h28]: 59.63%, respectively.

Comment [h29]: less inhibition (57.78, 55.56, and 51.11%), respectively.

Comment [h30]: exhibited no inhibition (0%).

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Comment [h31]: & Characterization of PGPR of Lentils Lens culinaris.

Comment [h32]: Check its presence in the literature?!

Comment [h33]: Check its presence in the literature?!

Comment [h34]: *Fusarium oxysporum* f. sp. lentis.

Comment [h35]: *Fusarium oxysporum* f. sp. lentis.

Comment [h36]: Italicize all the binomial names please.

Comment [h37]: Check its presence in the literature?!

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Comment [h38]: Check its presence in the manuscript pls?