International Journal of Plant & Soil Science

Volume 35, Issue 2, Page 7-11, 2023; Article no.IJPSS.96494
ISSN: 2320-7035

Biological Management of Lentil (*Lens culinaris* Medik) *Fusarium* wilt by Using the Potential *Pseudomonas* Isolates

Dilip Kumar Chaurasiya [a], Sangita Sahni [a*], Bishun Deo Prasad [b] and Birendra Kumar [a]

[a] Department of Plant Pathology, Dr Rajendra Prasad Central Agricultural University, Pusa, Samastipur-848125, Bihar, India.
[b] Department of AB&MB, CBS&H, Dr Rajendra Prasad Central Agricultural University, Pusa, Samastipur-848125, Bihar, India.

Authors' contributions

This work was carried out in collaboration among all authors. Author SS designed the research. Authors DKC and SS carried out the research and analyzed the data. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPSS/2023/v35i22752

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/96494

Original Research Article

Received: 21/10/2022
Accepted: 30/12/2022
Published: 07/02/2023

ABSTRACT

Lentil (*Lens culinaris Medik*) is also known as "massoor". *A member of the Leguminaceae family is known for their high protein and nutrient content.* Wilt incited by *Fusarium oxysporum* f.sp. *lentis* can result in significant yield losses upto 50% for lentil farmers. Chemical management through fungicides reduces crop losses significantly but has some environmental drawbacks and conflicts on the human health instead other methods of control such as the use of plant growth-promoting rhizobacteria (PGPR) as a bio-inoculant which gaining attention as a sustainable approach to manage plant diseases. PGPR are soil bacteria that live in the rhizosphere of plants and promote

**Corresponding author: E-mail: sangita@rpcau.ac.in, sangitampp@gmail.com;*

growth. The use of PGPR-based agents has increased in agriculture in recent years. In this study, isolation and evaluation of novel pseudomonad isolates were performed from various soil sources and exploited for controlling lentil wilt caused by *F. oxysporum* f.sp. *lentis* and assess their *in-vitro* ability to inhibit the pathogen.

Keywords: Lentil (Lens culinaris); Fusarium wilt; PGPR.

1. INTRODUCTION

The legume crops are unique due to their high protein content (27 – 34%) besides plenties of fiber, minerals, carbohydrates, and nutrients [1]. Lentil (*Lens culinaris* M.) is a deployed species (2n =14) self-pollinating crop which belongs to the family Leguminosae (*Fabaceae*), [2]. Wilt of lentil is incited by *Fusarium oxysporum* Schlecht. emend. Snyder & Hansen f. sp. *lentis* Vasudeva and Srinivasan (Fol) [3].

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% [4,5]. Pathogen can also survive within soil as chlamydospores, which can persist for several years [6]. 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 negligence in application of synthetic pesticides occurred in dismerits such as phytotoxicity, residues and risk to human and his environment as well [7]. 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 [8,9]. 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 [10]. 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 [11,12]. Due to PGPRs potential to manage soil-borne pathogens by colonizing plant roots [13] and detoxifying the environment, are considered as suitable solution for biological control [11,14,15]. 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.

2. MATERIALS AND METHODS

2.1 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 [16]. 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.

2.2 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 [10]. 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 [17].

2.3 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, where mycelium growth in the control and test plate is denoted by C and T, respectively, and I indicates the mycelial growth inhibition [18].

3. RESULTS AND DISCUSSION

3.1 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. 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, 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. This study are partially or fully go with the findings of Harsha et al. [19], 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.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 [14]. Similarly, twenty-four strains of *Bacillus sp.* (B4, B7 and B12) showed excellent antagonistic activity against investigated pathogenic fungi [11]. Experimental findings were also in accordance with previous results [20] that identified and characterized *Pseudomonas aeruginosa* with positive antagonistic potentials toward *Fusarium lycopersici* and demonstrated a radial inhibition of 67.85%. Isolation and antagonistic activity of rhizospheric bacteria against various disease-causing pathogens were also reported previously [21].

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*

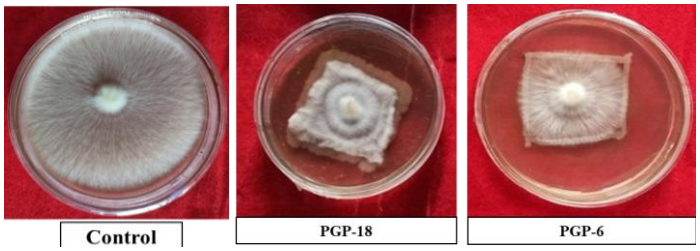

Fig. 1. Dual culture based screening of PGPRs (*Pseudomonad* isolates) against *F. oxysporum* f.sp. lentis (Fol) pathogen

4. CONCLUSION

In this study, the PGP18 isolate was found the most effective, among 20 isolates, and produced 67.41% inhibition against *F. oxysporium*. PGP6, PGP16, and PGP17 also showed significant inhibition with 61.85%, 61.48%, and 59.63%, respectively. PGP11, PGP15, and PGP20 showed less inhibition (57.78, 55.56, and 51.11%), respectively. The PGPR showing higher inhibion against *F. oxysporium* can be further exploited in making formulations for effective management of *Fusarium* wilt in lentil.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
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