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Characterization of Florescent Pseudomonads for Biological control Efficacy, Plant Growth Promotion and Antibiotic Tolerance

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

A collection of fourteen indigenous Fluorescent Pseudomonads (FLPs) was isolated from diverse crop rhizospheres across different regions of Bihar, and their distinctive characteristics were documented. The differentiation between *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* was achieved through pigmentation, fluorescence assessment on King's A media, and growth analysis at 42°C. Among the isolates, nine were identified as *P. aeruginosa*: FLP strains associated with Rice, Turmeric, Mustard-1, Mustard-2, Okra-G, Pea, Barley, Brinjal, and Chickpea. Meanwhile,

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five isolates - FLP Wheat, FLP Bean, FLP Mango, FLP Brinjal New, and FLP Cauliflower - were classified as *P. fluorescens*. Confirmation of these classifications was established through DNA sequencing of the 16s RNA region. These FLP isolates were evaluated against prevalent phytopathogens, including *Macrophomina phaseolina*, *Colletotrichum musae*, *Fusarium oxysporum* f. sp. *cubense*, and *Xanthomonas oryzae* pv. *oryzae*. Notable performance was exhibited by FLP Barley (70.55%) against *Macrophomina phaseolina*, FLP Okra-G (53.82%) against *Colletotrichum musae*, FLP Rice (38.58%) against *Fusarium oxysporum* f. sp. *cubense*, and FLP Okra-G against *Xanthomonas oryzae* pv. *oryzae*. In the context of biocontrol efficacy, FLP Turmeric, FLP Pea, FLP Okra-G, and FLP Mustard-1 showcased superior performance in combatting phytopathogens. Additionally, all FLP isolates, except FLP Cauliflower, exhibited phosphate-solubilizing capabilities, with FLP Brinjal demonstrating the highest solubilization (Phosphate Solubilizing Index of 2.89). Siderophore production was a common trait among the isolates. Compatibility with antibiotics, namely Streptocycline at concentrations of 0.025% and 0.10%, as well as Copper oxychloride at 0.25%, 0.30%, and 0.50%, was assessed. Only FLP Cauliflower exhibited compatibility with both antibiotics across all concentrations. Notably, FLP Rice, FLP Turmeric, and FLP Barley demonstrated positive antibiotic compatibility alongside effective biocontrol potential. In summation, this study unveils the distinct attributes and potential applications of indigenous Fluorescent Pseudomonads (FLPs) isolated from the rhizospheres of diverse crops in Bihar. The identified isolates exhibit promising traits such as disease control, phosphate solubilization, siderophore production, and antibiotic compatibility, thus presenting valuable options for sustainable agricultural practices and disease management.

Keywords: Florescent pseudomonads; biocontrol; PGPR; siderophore; phosphate solubilization.

1. INTRODUCTION

Plants and their antagonistic interaction with specific viruses, bacteria, fungi, nematodes, insects, arachnids, and weeds result in physiological stress, termed biotic stress. This complex mechanism of host-pathogen interaction wherein a plant is rendered susceptible, is a major cause of pre- and postharvest losses. The ability of any biotic stress factor to cause yield or quality loss depends on climate and environment, duration of interaction, and host-pathogen genomic constitution [1]. Plants lack an adaptive immune system, so they have evolved a range of sophisticated strategies to counteract biotic stresses. The plant immune and defense strategy is frequently amplified and augmented in the presence of beneficial Plant Growth Promoting Rhizobacteria (PGPR) [2]. In modern agroecological systems, the defense strategies of plants are supplemented by an array of agrochemicals like chemical fertilizers and pesticides. The use of effective management of pests and diseases has doubled food production in the past few decades, but despite efforts pathogens still claim 10-16% of the global harvest. Climate change has additionally begun to affect complex biological interactions of pest-pathogen and host, which necessitates integrated solutions and international coordination to make sense of the changing face of host-pathogen interplay [3]. Plant Growth

Promoting Rhizobacteria (PGPR) has the potential to replace the use of chemical fertilizers, pesticides, and other supplements in modern agriculture. Over the last decade, the use of PGPR has increased in agriculture owing to its eco-friendly and sustainable nature [4]. PGPR employ either a direct or indirect mechanism to regulate nutritional and hormonal balance in the plants, induce resistance against pathogens, solubilize nutrients for easy uptake by plants, and show synergistic as well as antagonistic interactions with other microorganisms in the rhizosphere [5]. They also secrete compounds that contain valuable biostimulants that modulate plant stress responses. Furthermore, PGPR have the capability to improve crop tolerance for abiotic stresses and can be instrumental as climate change conditions continue to develop [6].

Fluorescent Pseudomonads (FLPs) as PGPR have been shown to be beneficial to plants in countless ways. The host plant when inoculated with a potent rhizobacteria, can trigger a reaction in the plant roots, producing a signal that spreads systemically throughout the plant resulting in enhanced defensive capacity to subsequent pathogen infections. This reaction is very similar to pathogen-induced Systemic Acquired Resistance (SAR) and is called Induced Systemic Resistance (ISR) [7]. FLPs benefit the plant by producing secondary metabolites,

antibiotics, phytohormones, volatile compounds, and siderophores, act as fertilizer and pesticides, increase tolerance to abiotic stress, etc. Kloepper et al. [8] put forward the idea that Pseudomonads have the capability to use heterologous siderophores which gives them a competitive advantage over other fungal and bacterial pathogens in the plant rhizosphere. It is also known that the efficiency of phosphatic fertilizers in India is very low due to their fixation in both acidic and alkaline soils, hence, phosphate solubilizing bacteria (PSB) increase yield when inoculated alone or in combination with rock phosphate and mycorrhiza. They increase nodulation in legumes and enhance N-uptake [9]. Phenazine produced by Pseudomonads, possess redox activity, and can suppress pathogens such as *F. oxysporum* and *Gaeumannomyces graminis* [10]. Cantore PL et al. [11] indicated the presence of an array of volatiles such as ammonia, HCN, and dimethyl disulphide, produced by Pseudomonads that can inhibit the growth of phytopathogenic fungi.

Numerous such studies present strong evidence for the promise that FLPs provide against modern agricultural problems of degrading soil quality and diversity, pollution caused by agriculture, and new and emerging pathogens and pests. In addition to addressing the above problems, PGPR also associate with plants to increase their growth, yield, and stress tolerance. The study was taken up with the intention to identify the local FLPs suitable to use in the fields of farmers, provide them with the benefits of effective PGPR and promote sustainable agriculture.

2. MATERIALS AND METHODS

2.1 Isolation, Growth, Characterization of Isolates

The FLPs were isolated from the rhizosphere of 14 commonly grown crops from different locations Bihar. The preliminary differentiation between the various isolates was done by observing their growth and fluorescence on King's A media, King's B media and at 42 °C [12].

2.2 Phosphate Solubilization

The phosphate solubilizing capacity of the isolates was qualitatively determined using Pikovaskaya media, which was prepared using Tricalcium phosphate as the phosphate source

[13]. Small circular paper discs of about 3 mm diameter were dipped into the bacterial broth and placed onto the center of the plate and observed for halo production [13].

$$\text{Phosphate Solubilisation Index (PSI)} = \frac{C(\text{colony diameter})+H(\text{halo diameter})}{C}$$

2.3 Siderophore Production

Chrome azurol S was added in King's B media base to prepare the CAS agar media. Bacteria was inoculated by touching a loopful onto the center of Petri-plate and observed for orange halo. [14].

$$\text{Siderophore Production Index (SPI)} = \frac{C(\text{colony diameter})+H(\text{halo diameter})}{C}$$

2.4 Antifungal and Antibacterial Assay

An anti-fungal assay was done against *Fusarium oxysporium* f. sp. *cubense*, *Colletotrichum musae*, and *Macrophomina phaseolina*. On a PDA media plate, a 3 mm disc of fungus was placed about 1 cm away from the rim of the Petri-plate and the bacteria was streaked in a single straight-line opposite to it (Dennis and Webster, 1971). The distance between the furthest fungal growth point and the bacterial streak was measured.

$$\text{Percent inhibition} = \frac{(C(\text{growth in control})-T(\text{growth in treatment}))}{C} \times 100 \quad [15]$$

Antibacterial Assay was done against *Xanthomonas oryzae* pv. *oryzae* (Xoo) on NA media. Xoo and Pseudomonad isolates were streaked in single straight-line perpendicular and intersecting to each other in the center of the Petri-plate. The presence of a halo zone at intersection is indicative of control by isolates. Diameter of the halo zone (if present) was measured to quantify the level of control [16].

$$\text{Percent inhibition} = \frac{(C(\text{width or halo in control})-T(\text{width or halo in treatment}))}{C} \times 100$$

2.5 Assay for Compatibility with Antibacterial Agents

Streptocycline at 0.025% (250 ppm) and 0.10% (1000 ppm) and COC at 0.25% (2500 ppm), 0.30% (3000 ppm) and 0.50% (5000 ppm) were

used for an assay for compatibility with antibacterial agents. Sterilized discs of filter paper of about 3 mm diameter were soaked in antibacterial solutions and placed over the King's B Petri-plate inoculated with different isolates. The diameter of the halo zone around the disc was measured to quantify the level of control/incompatibility by the antibacterial agent.

2.6 Molecular Identification

2.6.1 Genomic DNA extraction

10mL of each bacterial strain was grown in King's B broth at 30°C for 24 hrs. About 1 mL of the grown bacterial culture was taken and subjected to DNA extraction using a DNA extraction kit. DNA was checked for quality and quantity by 0.5 % agarose gel electrophoresis using uncut lambda (λ) DNA as standard.

2.6.2 Polymerase Chain Reaction (PCR) and sequencing

PCR amplification of the 16s rDNA ITS region was done using the universal primers 27F (AGAGTTTGATCMTGGCTCAG) and 1492R (TACGGYTACCTTGTTACGACTT) in a thermocycler. The amplification program used was initial denaturation (5 minutes at 94°C), followed by 35 cycles each comprising denaturation steps (35 Sec. at 94 °C), a step of annealing (30 Sec. at 50 °C) an elongation step (30 Sec. at 72 °C) and a final elongation for 10 minutes at 72°C.

3. RESULTS AND DISCUSSION

3.1 Characterization of Indigenous Pseudomonad Isolates

The color on King's B media was fairly uniform, varying only between a bright to a lesser bright green. As opposed to that, the colour on King's A medium had significant variation among the isolates. FLP; Wheat, Bean, Mango, Brinjal New, and Cauliflower produced a putrid smell while FLP; Rice, Turmeric, Mustard-1, Okra-G, Pea, and Barley produced a fruity smell. No strong smell was detected in FLP; Mustard-2, Brinjal and Chickpea. The isolates which produced blue-green pigmentation on King's A media also gave a fruity smell (except FLP; Mustard-2 and Brinjal which had no strong smell) which is a characteristic of *P. aeruginosa* [17]. The remaining isolates (except Chickpea which had no strong smell) gave a putrid smell which is identified to be a characteristic of *P. fluorescens*.

Among the thirteen isolates only five isolates namely, FLP; Wheat, Bean, Mango, Brinjal New and Cauliflower did not grow at 42°C and hence were concluded to be *P. fluorescence* and the rest were concluded to be *P. aeruginosa*. The results of the present investigation are in line with that of La Bauve and Wargo [18] who used King's A medium to observe pyocyanin pigmentation in *Pseudomonas* spp. and used temperatures ranging from 4°C to 42°C to differentiate between various Pseudomonad isolates. Through laboratory experiments, ten out of a total of fifteen isolates were found to be *P. aeruginosa* (66.66%). This identification was further confirmed after gene sequencing and BLAST analysis. *P. aeruginosa* isolates were also more vigorous in growth, grew faster and greater in number, and were more fluorescent on King's B media in comparison to *P. fluorescens*.

3.2 Biocontrol of Fungal Phytopathogens

Macrophomina phaseiolina showed complete growth within 4 days of inoculation into the control plate, *Colletotrichum musae* took 8 days while *F. oxysporum* f. sp. *ubense* showed complete growth within 12 days. Eight isolates did not show any inhibition in the growth of *Macrophomina phaseiolina*, namely, FLP; Brinjal, Wheat, Bean, Chickpea, Mango, Brinjal New and Cauliflower. FLP Barley showed the maximum inhibition and FLP Okra G and FLP Pea were at par with each other. The isolates which showed inhibition are FLP; Barley, Okra-G, Pea, Mustard-1, Mustard-2, Turmeric, and Rice with inhibition per cent of 70.55%, 61.04%, 55.01%, 52.70%, 49.41%, 41.89%, and 32.43% respectively. FLP; Wheat, Bean, Mango, Brinjal New, and Cauliflower could not inhibit *Colletotrichum musae* while FLP; Okra-G, Turmeric, Pea, Barley, Rice, Mustard-2, and Mustard-1 showed an inhibition per cent of 54.12%, 49.41%, 49.19%, 48.80%, 46.99%, 44.88%, and 42.69%, respectively. FLP Okra-G showed maximum inhibition and the rest of the isolates are at par with each other. Three of the 15 isolates inhibited further growth of fungi only after the fungal mycelium came in contact with the bacterial streak; FLP; Brinjal and Chickpea (Table 1).

In the case of *F. oxysporum* f. sp. *ubense*, five isolates did not show any inhibition in fungal growth namely, FLP; Brinjal, Wheat, Bean, Mango, and Brinjal New while FLP; Rice, Pea, Turmeric, Okra-G, Mustard-1 and Barley showed an inhibition of 38.93%, 38.61%, 35.50%,

30.75%, 30.12%, 28.54%, and 28.29%, respectively. FLP Brinjal and FLP Turmeric are at par with each other. FLP; Mustard-2, Chickpea, and Cauliflower inhibited further growth of fungi only after the mycelium came in contact with the bacterial streak (Table 1).

It is evident that most isolates could not effectively control *Macrophomina phaseolina* probably because of being the faster growing fungi. None of the *P. fluorescens* isolates inhibited *Colletotrichum musae*. *P. fluorescens* isolates also did not perform well when compared to *P. aeruginosa* in case of *F. oxysporum* f. sp. *cubense*. Out of fourteen, only a

few isolates were effective against all three fungi which were FLP; Barley, Pea, and Okra-G, all three of them are *P. aeruginosa*. The study is in conformity with that of Bakthavatchalu et al. [19] who found that *P. aeruginosa* controlled *Colletotrichum gloeosporioides* up to 72.1%. In a similar experiment, Karimi et al. [20] tested *P. aeruginosa* and *P. putida* against *Fusarium oxysporum* f. sp. *cicero* by three methods; in dual culture method *P. aeruginosa* showed 45% inhibition and *P. putida* 40% inhibition, in extracellular metabolites method the inhibition was 43% and 38%, and for the volatile compound method, it was 26%, 23% respectively.

Table 1. Biocontrol efficacy of fluorescent pseudomonad isolates

S No.	Isolates	Inhibition of			Max. width of Xoo streak (cm)	Halo produced (cm)
		<i>M. phaseolina</i> (%)	<i>C. musae</i> (%)	FOC (%)		
1	Rice (<i>P. aeruginosa</i>)	32.43 ^e (34.71)	46.99 ^b (42.53)	38.93 ^a (38.60)	0.85 ^b	1.05 ^c
2	Turmeric (<i>P. aeruginosa</i>)	41.89 ^d (40.30)	49.41 ^b (44.52)	35.50 ^b (36.57)	0.55 ^a	1.05 ^c
3	Muatar-2 (<i>P. aeruginosa</i>)	49.41 ^c (44.66)	44.88 ^b (42.21)	28.11 ^d (32.16)	1.00 ^b	0.00 ^e
4	Mustard-1 (<i>P. aeruginosa</i>)	52.70 ^c (46.55)	42.69 ^b (40.76)	30.13 ^c (33.29)	0.68 ^a	1.75 ^b
5	Okra-G (<i>P. aeruginosa</i>)	61.04 ^b (51.38)	54.12 ^a (47.70)	30.76 ^c (33.68)	0.93 ^b	2.15 ^a
6	Pea (<i>P. aeruginosa</i>)	55.07 ^b (47.92)	49.19 ^b (44.52)	38.61 ^a (38.41)	0.83 ^b	1.70 ^b
7	Barley (<i>P. aeruginosa</i>)	70.55 ^a (57.13)	48.80 ^b (43.18)	28.11 ^d (32.19)	0.90 ^b	0.25 ^e
8	Brinjal (<i>P. aeruginosa</i>)	0 ^f (0)	28.11 ^c (32.158)	0 ^e (0)	0.60 ^a	0.60 ^d
9	Wheat (<i>P. fluorescens</i>)	0 ^f (0)	0 ^d (0)	0 ^e (0)	0.80 ^b	0.00 ^e
10	Bean (<i>P. fluorescens</i>)	0 ^f (0)	0 ^d (0)	0 ^e (0)	0.80 ^b	0.00 ^e
11	Chickpea (<i>P. aeruginosa</i>)	0 ^f (0)	28.11 ^c (32.158)	28.11 ^d (32.158)	0.73 ^a	0.00 ^e
12	Mango (<i>P. fluorescens</i>)	0 ^f (0)	0 ^d (0)	0 ^e (0)	0.77 ^a	0.00 ^e
13	Brinjal New (<i>P. fluorescens</i>)	0 ^f (0)	0 ^d (0)	0 ^e (0)	0.60 ^a	0.00 ^e
14	Cauliflower (<i>P. fluorescens</i>)	0 ^f (0)	0 ^d (0)	28.11 ^d (32.158)	0.80 ^b	0.00 ^e
15	Control	0 ^f (0)	0 ^d (0)	0 ^e (0)	1.30 ^c	0.00 ^e
	SEm	1.01	0.71	0.23	0.06	0.08
	CD (0.01)	3.91	2.74	0.87	0.23	0.33
	CV%	8.70	4.90	1.80	12.60	31.80

It was observed that a few of the isolates like FLP Chickpea could limit fungal growth only after the FLP came in contact with the fungal pathogens used in the experiment, i.e., fungal mycelium touching the FLP streak led to the inhibition of fungal mycelium growth. In contrast to this, the fungi grew right over the bacterial streak in the instance where the FLPs were ineffective in controlling the fungal growth. This pattern is indicative of the presence of at least two different methods of control of fungi. A plethora of control methods are recorded to be used by Pseudomonads, many of which are simultaneously working at any given time against the phytopathogens. Cantore PL et al. [11] indicated the presence of an array of volatiles such as ammonia, HCN, and dimethyl disulphide which are produced by Pseudomonads that can inhibit the growth of phytopathogenic fungi. Phenazines inhibit electron transport and might have the ability to solubilize iron, HCN inhibits metalloenzymes, especially copper-containing cytochrome oxidases, etc. [21,22].

3.3 Biocontrol of Bacterial Phytopathogen

While the width of the FLP streak was normal throughout the plate, the width of Xoo streak decreased at the point of intersection of Xoo and FLP isolates. The maximum streak width of Xoo was the least in FLP Turmeric (0.55cm). Halo was observed at the point of intersection of Xoo and FLP in only 7 isolates. It was maximum in FLP Okra-G being 2.15 cm, followed by FLP Mustard-1 at 1.75 cm, FLP Pea at 1.70 cm, FLP Rice and FLP Turmeric at 1.05 cm, FLP Brinjal at 0.60 cm and FLP Barley (0.25 cm). A vast distinction in control and treatment was not observed in the Pseudomonad control of Xoo. Some FLPs produced a halo at the point of bacterial intersection and all FLPs showed a reduction in the width of the Xoo streak. It is noted here that there is no correlation between the reduction of the width of the Xoo streak and the production of a halo among the isolates, some isolates which produced no halo significantly reduced the width of the Xoo streak (Table 1).

3.4 Phosphate Solubilization and Siderophore Production

All isolates were found to be phosphate solubilizing except Cauliflower. Maximum Phosphate solubilization was seen in FLP Chickpea with 3.23 PSI and FLP Brinjal with 3.0,

FLP Turmeric with 2.93 and FLP Rice with 2.73. FLP; Mustard-2, Mustard-1, Pea, Barley, Okra-G, Bean, and Wheat had a PSI of 2.68, 2.46, 2.30, 2.12, 2.07, 1.50, 1.19, respectively. FLP Brinjal New and FLP Mango had the least PSI of 1.11 and 1.08, respectively. All 14 FLP isolates produced siderophore. Maximum siderophore production was seen in FLP Cauliflower with 2.23 SPI, closely followed by FLP Turmeric and FLP Okra-G with 2.13 SPI. They were followed by FLP; Barley, Rice, Pea, Brinjal New, Mustard-1, Brinjal, Mustard-2, Wheat, and Chickpea with siderophore production index (SPI) of 2.1, 2.0, 1.97, 1.8, 1.77, 1.70, 1.77, 1.63, 1.50, 1.33 respectively. FLP Mango and FLP Bean had the least SPI of 0.57 and 0.53, respectively. (Table 2; Figs. 2 and 3)

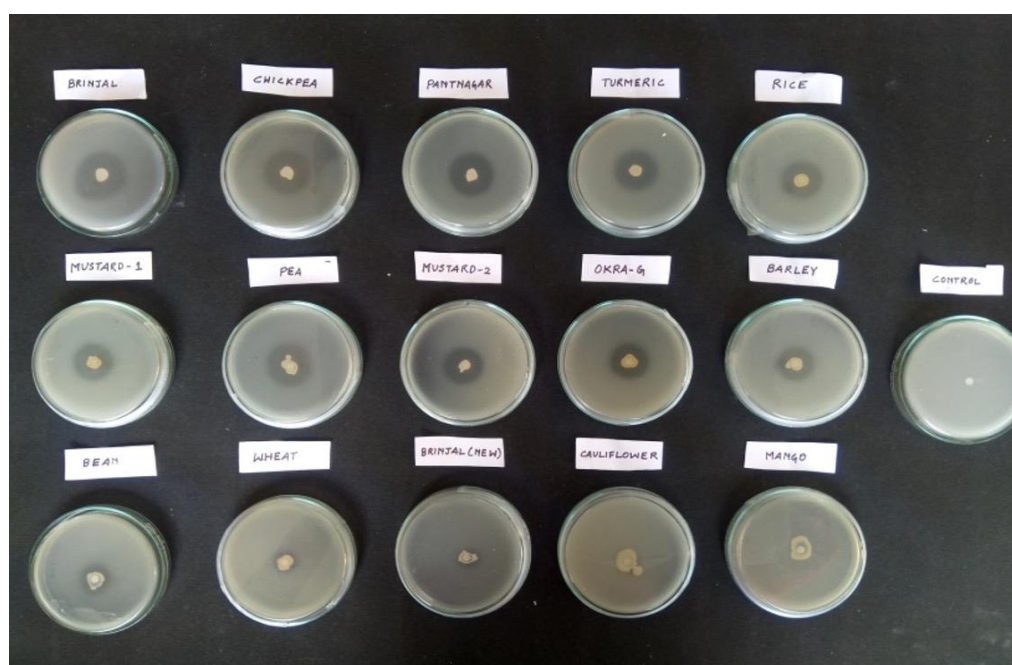
It is evident that FLP; Rice, Mustard-1, Mustard-2, Okra, Pea, Barley, and Turmeric are capable of producing siderophore, solubilizing phosphate, and are also effective as biocontrol. A critical review of the available literature suggests that the production of phosphate and siderophore can be correlated with biocontrol activity. Janardan and Verma [23] observed that among the eight Pseudomonad isolates (rice rhizosphere) strains showing higher phosphate solubilization also recorded greater growth inhibition of *Rhizoctonia solani*. A bacterial strain with high phosphate solubilizing capacity was found to be genetically closer to *P. aeruginosa* and was an antagonist to *E. coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Vibrio cholerae*, *Micrococcus luteus* [24]. It was noticed that purified pyoverdine-negative mutants of strain *Pseudomonas* sp. WCS417r and *P. putida* WCS358r could not control wilt in Carnation [25,26].

3.5 Compatibility of Pseudomonad Isolates with Commonly Used Antibiotics

All the Pseudomonad isolates were completely compatible with streptomycin at 0.025%, except Mustard-2, whose growth was slightly reduced. At streptomycin concentration of 0.10% Barley and Cauliflower showed complete compatibility and no inhibition in growth. For Rice and Wheat, growth was reduced only slightly by 0.05 cm, followed by Brinjal and Chickpea which were inhibited by 0.10 cm, Mustard-2 with 0.15 cm inhibition, Okra-G and Pea with 0.20 cm, Brinjal New with 0.25 cm, Bean and Mango with 0.35 cm and maximum in Mustard-1 and Turmeric with 0.40cm (Table 3; Fig. 3).

Table 2. Compatibility of fluorescent pseudomonad isolates with commercial agrochemicals at recommended dosage

S No.	Isolates	Inhibition (cm) by				
		Streptocycline 0.025%	Streptocycline 0.10%	COC 0.25%	COC 0.30%	COC 0.50%
	Rice (<i>P. aeruginosa</i>)	0 ^a	0.05 ^a	0.00 ^a	0.00 ^a	0.00 ^a
	Turmeric (<i>P. aeruginosa</i>)	0 ^a	0.40 ^b	0.00 ^a	0.00 ^a	0.00 ^a
	Muatar-2 (<i>P. aeruginosa</i>)	0 ^a	0.15 ^b	0.25 ^b	0.40 ^c	0.70 ^d
	Mustard-1 (<i>P. aeruginosa</i>)	0.1 ^b	0.40 ^b	0.80 ^c	1.40 ^e	1.60 ^f
	Okra-G (<i>P. aeruginosa</i>)	0 ^a	0.20 ^b	0.00 ^a	0.20 ^b	0.50 ^c
	Pea (<i>P. aeruginosa</i>)	0 ^a	0.20 ^b	0.00 ^a	0.60 ^d	0.40 ^c
	Barley (<i>P. aeruginosa</i>)	0 ^a	0.00 ^a	0.00 ^a	0.05 ^a	0.15 ^b
	Brinjal (<i>P. aeruginosa</i>)	0.05 ^a	0.10 ^a	0.30 ^b	0.50 ^d	1.00 ^e
	Wheat (<i>P. fluorescens</i>)	0 ^a	0.05 ^a	0.00 ^a	0.15 ^d	0.25 ^b
	Bean (<i>P. fluorescens</i>)	0 ^a	0.35 ^b	0.00 ^a	0.00 ^a	0.00 ^a
	Chickpea (<i>P. aeruginosa</i>)	0.05 ^a	0.10 ^a	0.15 ^b	0.45 ^d	0.70 ^d
	Mango (<i>P. fluorescens</i>)	0.05 ^a	0.35 ^b	0.10 ^b	0.45 ^d	0.35 ^c
	Brinjal New (<i>P. fluorescens</i>)	0 ^a	0.25 ^a	0.00 ^a	0.05 ^d	0.15 ^b
	Cauliflower (<i>P. fluorescens</i>)	0 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a
	Control	0 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a
	SEm	0.022	0.059	0.054	0.037	0.045
	CD (0.01)	0.089	0.242	0.225	0.154	0.186
	CV%	293.9	76.50	112.10	27.40	24.50

**Fig. 1. Phosphate solubilization index of FLP isolates under Pikovaskaya media**

When tested with copper oxychloride at a concentration of 0.25%, FLP; Rice, Turmeric, Okra-G, Pea, Barley, Wheat, Bean, Brinjal New, and Cauliflower showed complete compatibility. FLP Mango was inhibited by 0.10 cm followed by FLP; Chickpea, Mustard-2, Brinjal, Mustard-1 at 0.15 cm, 0.25 cm, 0.30 cm

and 0.80 cm inhibition in growth. At copper oxychloride concentration of 0.30% FLP; Rice, Turmeric, Bean, and Cauliflower showed complete compatibility. For FLP Brinjal New and FLP Barley growth was reduced by 0.05 cm, followed by FLP; Wheat, Okra-G, Mustard-2, Chickpea, Mango, Brinjal, Pea and Mustard-1

isolates at 0.15 cm, 0.20 cm, 0.40 cm, 0.40 cm, 0.45cm, 0.45cm, 0.50 cm, 0.60 cm, and 1.40 cm, respectively. Copper oxychloride at a concentration of 0.50% showed complete compatibility with FLP; Rice, Turmeric, Bean, and Cauliflower. For FLP Barley and FLP Brinjal New

growth was reduced by 0.15 cm, followed by FLP; Wheat, Mango, Pea, Okra-G, Mustrad-2, Chickpea, Brinjal, Mustard-1 at 0.25 cm, 0.35 cm, 0.40 cm, 0.45 cm, 0.50 cm, 0.70 cm, 0.70 cm, 1.00 cm, and 1.60cm growth reduction (Table 3; Fig. 4).

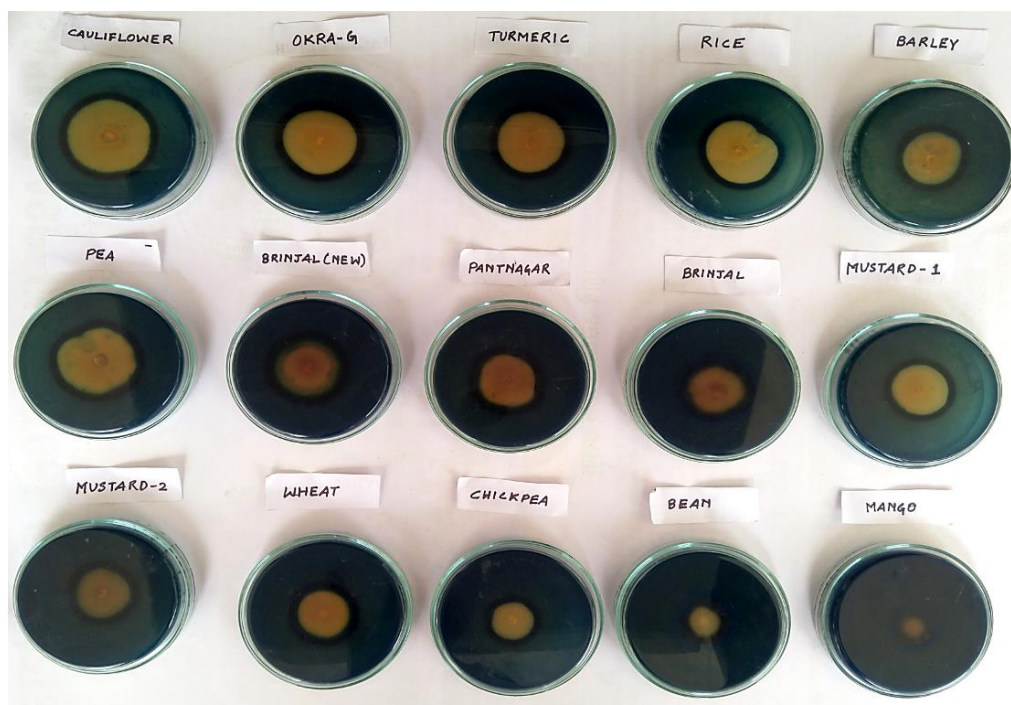


Fig. 2. Siderophore production index of FLP isolates grown on King's B media with chromoazurol S dye

Table 3. Phosphate solubilization index and siderophore production index of different fluorescent pseudomonad isolates

S No.	Isolates	PSI	SPI
1	Rice (<i>P. aeruginosa</i>)	2.73 ^c	2.00 ^b
2	Turmeric (<i>P. aeruginosa</i>)	2.93 ^b	2.13 ^a
3	Muatarad-2 (<i>P. aeruginosa</i>)	2.68 ^c	1.63 ^c
4	Mustard-1 (<i>P. aeruginosa</i>)	2.47 ^c	1.77 ^c
5	Okra-G (<i>P. aeruginosa</i>)	2.07 ^d	2.13 ^a
6	Pea (<i>P. aeruginosa</i>)	2.30 ^d	1.97 ^b
7	Barley (<i>P. aeruginosa</i>)	2.12 ^d	2.10 ^a
8	Brinjal (<i>P. aeruginosa</i>)	3.00 ^b	1.70 ^c
9	Wheat (<i>P. fluorescens</i>)	1.19 ^e	1.63 ^c
10	Bean (<i>P. fluorescens</i>)	1.50 ^e	0.53 ^e
11	Chickpea (<i>P. aeruginosa</i>)	3.23 ^b	1.13 ^d
12	Mango (<i>P. fluorescens</i>)	1.08 ^f	0.57 ^e
13	Brinjal New (<i>P. fluorescens</i>)	1.11 ^f	1.80 ^b
14	Cauliflower (<i>P. fluorescens</i>)	1.00 ^g	2.23 ^a
15	Control	1.00 ^f	0.00 ^f
	SEm	0.097	0.055
	CD (0.01)	0.377	0.211
	CV%	8.200	6.100

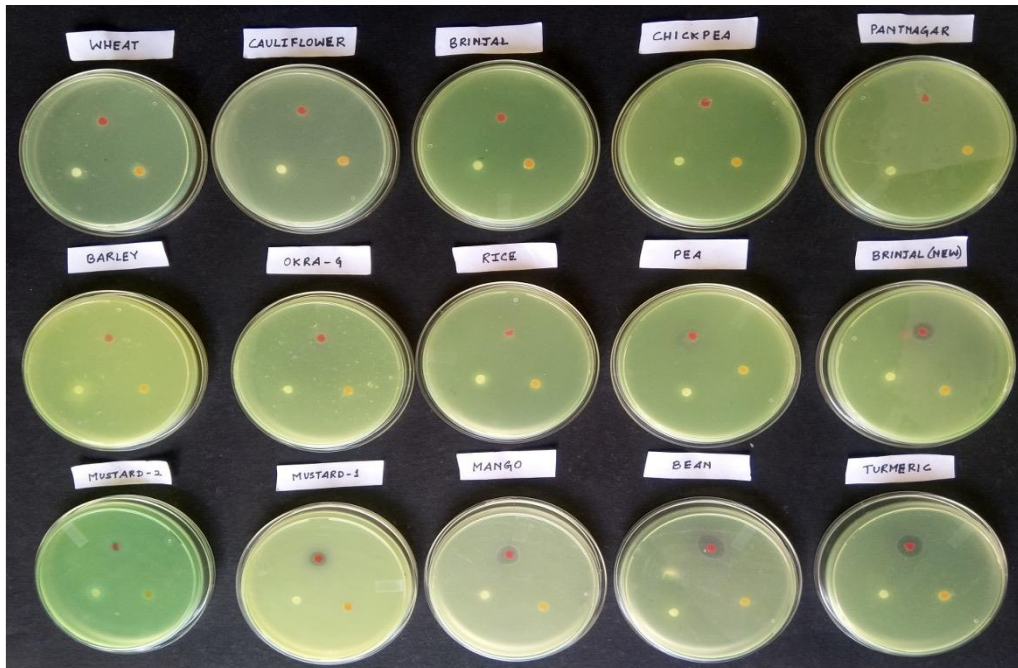


Fig. 3. Growth of *FLP* isolates at different concentrations of streptocycline (0.025% (yellow) and 0.1% (red), control is white)

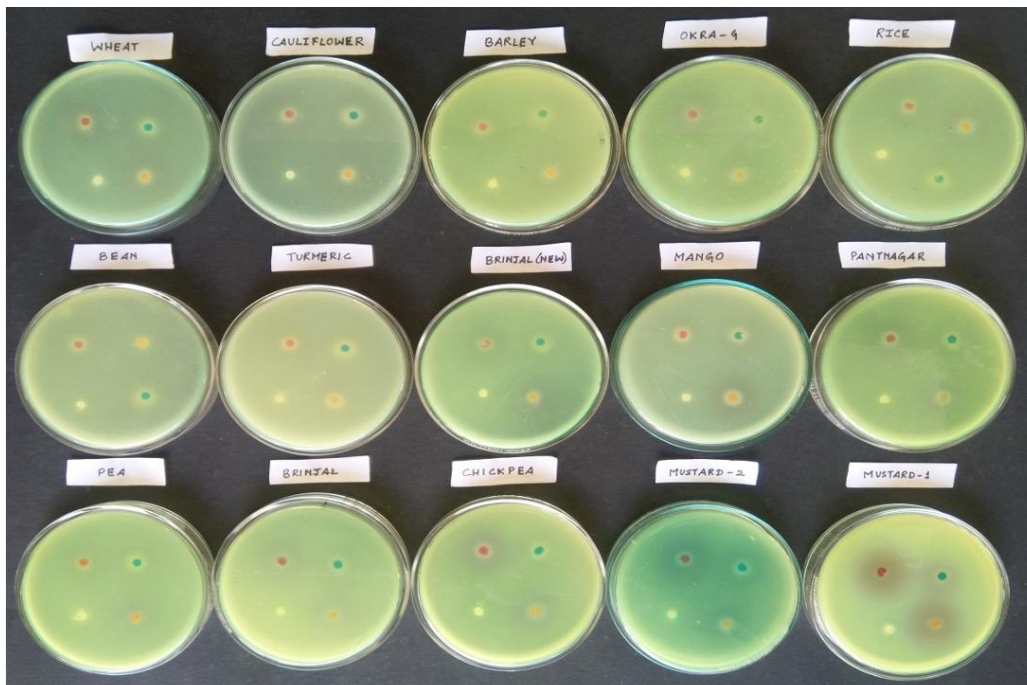


Fig. 4. Growth of *FLP* isolates at different concentrations of copper oxychloride (0.025% (yellow) and 0.1% (red), control is white)

Integrated Pest Management (IPM) is becoming increasingly important in agriculture to increase subsistence and maintain ecological balance, wherein the various components of fungicides, insecticides, and bioagents are integrated and

put into use. Hence, an analysis of the compatibility of these bioagents with that of some antibiotics was done. In the present investigation, it was found that all the *Pseudomonad* isolates were completely compatible with streptocycline

at 0.025 per cent, which is the most commonly recommended dosage in agriculture. Streptocycline at higher dosage of 0.1 per cent was also compatible with some FLPs like Barley and Cauliflower. When pseudomonad isolates were tested with copper oxychloride at a concentration of 0.25 per cent, which is the commonly recommended dosage, FLP; Rice, Turmeric, Okra-G, Pea, Barley, Wheat, Bean, Brinjal New and Cauliflower showed complete compatibility and no inhibition in growth. FLP; Rice, Turmeric, Cauliflower and Bean were compatible with COC at all concentration used in the test. Only FLP Cauliflower was compatible with both agrochemical at all the concentrations used in this experiment. Streptocycline and COC are commonly recommended in conjunction against all bacterial diseases so compatibility with these agrochemicals means that we can use them in integration with other methods of control.

Effective antagonists are typically identified only after screening large collections of isolates for plant disease suppression, indicating that only a subset of strains within the Pseudomonad group provide biological control. The reason for the availability of fewer effective PGPRs is that most biocontrol genes are located in the variable region of the genome and hence is not present across all the species [27,28,29].

4. CONCLUSION

The study was undertaken with the goal of identifying local FLPs suited for use in farmer fields, providing them with the benefits of effective PGPR, and promoting sustainable agriculture. PGPR has the potential to replace the use of chemical fertilizers, pesticides, and other supplements in modern agriculture. This work reveals the different characteristics and possible applications of indigenous FLPs isolated from the rhizospheres of various crops in Bihar.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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