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In-vitro and *In-vivo* Biocontrol potential of Florescent Pseudomonads against Major Diseases of Tomato

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ABSTRACT: Growing use of chemicals under high value vegetable crops tomato for pest and disease management is one of the prime concerns of food safety. Scientific identification and deployment biocontrol agents are a promising alternative management strategy for sustainable pest and disease management. In this context, characterization native microbial strains will be an important step towards successful biocontrol programme. Five indigenous Fluorescent Pseudomonads (FLPs) were isolated from the rhizosphere of various crops, *viz*., Rice, Okra, Barley, Brinjal and Chickpea from different locations of Bihar. *In-vivo* and *in-vitro* studies were conducted on five isolates namely *FLP; Rice*, *Okra*, *Barley*, *Brinjal New*, and *Chickpea*, against *Fusarium oxysporum* f.sp. *lycopersici* (FOL) and *Alternaria alternata*, *Alternaria solani,* causing wilt, brown spot and early blight respectively. Maximum percent of inhibition in the growth of fungi in dual culture was recorded by FLP Brinjal New (37.64%), FLP Rice (41.78%), and FLP Brinjal New and FLP Barley (37.64%), respectively. Seed bacterization with FLPs using a talc based FLP formulation increased germination percentage, days to 50 per cent germination, and seedling vigour. In the paper towel method of germination testing, FLP Brinjal New bacterized seeds took the least number of days (4 days) to 50 per cent seed germination and maximum germination percent was observed under FLP rice (96.33%). When bacterized seeds were planted in portrays, maximum germination was shown by FLP Brinjal New (95.33) while the least germination was recorded under untreated control and FLP rice (89.33). Maximum shoot length, root length, and seedling length were for bacterized seedlings of FLP Rice, FLP barley, and FLP Rice respectively. Two tomato pathogens, *Alternaria solani* causing early blight and *Fusarium oxysporum* f.sp. *lycopersici* causing wilt, were inoculated on the seedlings of FLP bacterized tomato. The minimum disease index against *Alternaria solani* was found under FLP Barley (17.96%) and against *F. oxysporum* f.sp. *lycopersici*, FLP Rice (44.71%) performed better than the other isolates. Among the seedling challenged with FOL, the earliest seed germination and maximum germination percentage were observed under seed bacterized with FLP Barley. Shoot length, root length, seedling length, and seedling vigor were maximum for FLP Rice bacterized seedlings.

Keywords: Biocontrol, Florescent Pseudomonad, Fusarium wilt, Seed bacterization, Tomato.

INTRODUCTION

The bacterial PGPR mostly belong to genus Pseudomonas and *Bacillus* spp., and are widely recognized as antagonists of root pathogens. Pseudomonads belong to the genus Pseudomonas, family *Pseudomonadaceae*, *Pseudomonadales*, class *Gammaproteobacteria* and phylum *Proteobacteria*. There are about 191 described species in this genus. Pseudomonads are the most diverse and ecologically significant group of bacteria on the planet, are ubiquitous, survive harsh conditions and form intimate associations with both plants and animals (Spiers *et al*., 2000). Fluorescent Pseudomonads (FLPs) as PGPR have been shown support plants in biotic and biotic stress. The host plant when inoculated with a potent FLP trigger a reaction in the plant roots leading to a signal that spreads systemically throughout the plant, finally resulting in enhanced defensive capacity to subsequent pathogen infections. This reaction in very similar to pathogen induced SAR and is called ISR. (Leeman *et al*., 1995b). Induced Systemic Response (ISR) was formerly described by Van Peer *et al.* (1991) in carnation plants that were systemically protected by the *P. fluorescens* strain WCS417r against *F. oxysporum* f. sp. *dianthi*. Treatment of tobacco roots with *P. fluorescens* CHA0 was found to trigger an accumulation of SA-inducible PR proteins in the leaves which protects the plants from phytopathogens (Maurhofer *et al*., 1994).

Plants create a microenvironment suitable for beneficial bacteria by releasing organic compounds creating a selective environment with less diversity. PGPR benefit the plant by producing secondary metabolites, antibiotics, phytohormones, volatile compounds, siderophores, act as fertilizer and pesticides, increase tolerance to abiotic stress, etc. Major bottle neck in use of Pseudomonads as biocontrol is its inability to produce resting spores thus making it difficult to formulate (Shivsakthi *et al*., 2014).

FLPs along with providing protection for stress also supplies nutrition to the plants. Fertilizers and pesticides, though vital tools for worldwide food safety, have become an unavoidable threat to agriculture. Continuous use of chemical fertilizers has proven to be responsible for the decline in soil organic matter content, decrease in the quality of agricultural soil, reduction in soil fertility, pollution of air, water, and soil, weakened microbial activity, altered soilpH, increased pests and diseases, acidification, decrease in population of useful organisms and soil biodiversity, etc (Pahalvi *et al*., 2021). Soil acidification is a result of the increased use of acidifying nitrogen fertilizers. One study by Lin *et al.* (2019) used RDA analysis to show that acidic microbes present in acidic soils were associated with heavy metals which in turn affect the desirable microbial community structure, microbial biomass, and microbial residues. Additionally, pesticide contamination can cause agrochemical pollution, microbial diversity loss, and a decline in the abundance of terrestrial invertebrates (Gunstone *et al*., 2021). Lin *et al*. (2019) found that the use of organic fertilizers containing the adequate amount of N, P, K restored soil health and microbial diversity while tackling the issue of heavy metal pollution. Many such studies have indicated a much-needed shift to organic and more eco-friendly methods of supplementing modern agriculture (Bisht and Chauhan 2020; Hussain *et al*, 2009; Onwona-Kwakye *et al*, 2020). Singh *et al*. (2018) emphasized the need to encourage and integrate organic farming in eastern India to increase farm income and sustainability along with the need for exploration and research for effective location specific microbial strain to overcome the bottle-neck in organic farming.

METHODS AND MATERIALS

Antifungal and antibacterial assay. An anti-fungal assay for all isolates was done *Fusarium oxysporum* f.sp. *lycopersici*, *Alternaria alternata*, and *Alternaria solani* on PDA media. Under aseptic conditions, a 3 mm disc of fungus was placed about 1cm away from the rim of the Petri-plate and the bacteria was streaked in a single straight-line opposite to it, making the approximate distance between the streak and fungal disc 5 cm on a Petri-plate of 7 cm diameter (Dennis and Webster 1971).

$$\text{Percent inhibition} = \frac{\text{C(growth in control)} - \text{T (growth in treatment)}}{\text{c}} \times 100 \quad \text{(Vincent, 1947)}$$

Seed bacterization and blotting paper analysis. About 50 seeds of tomato (*Lycopersicon esculentum* Mill.) cultivar Pusa Vishal were inoculated with 0.5gram talc-based FLP inoculum with CFU of 10^7mL^{-1} for each FLP isolate. The bacterized seeds were spread on sterile wet Blotting paper and allowed to germinate in a growth chamber maintained at 28°C and 75% relative humidity (RH) at 12-hour light period and 12-hour dark period. Sterile distilled water was sprinkled regularly on blotting paper to sustain the germination process for 10 days.

Evaluation of the efficiency of FLP as PGPR and biocontrol agent. For the purpose of evaluating the efficacy of FLPs as PGPR, seeds of tomato (cultivar Pusa Vishal) were bacterized with FLP and placed in a portray and were measured for germination percentage, shoot length, root length, and seedling length. The seedlings were allowed to grow for 28 days along with an un-inoculated control to check for their efficacy as PGPR.

To assess their efficacy as a biocontrol agent the seeds were inoculated with FLP + pathogen. FLP were tested against *Fusarium oxysporum* f.sp. *lycopersici*(FOL) and *Alternaria solani.* 100 mL suspension of FOL of concentration of 10^6spores mL^{-1} was used per portray (50 seedlings) and the bacterized seeds were then placed in each cavity. On the other hand, bacterized seeds grown in portrays were inoculated by foliar spray of *Alternaria solani*at 10^6spores mL^{-1} concentration at 21 days old plant stage or at the third leaf stage by leaf spray with equal and sufficient volume to ensure that all leaves are wet with conidia suspension. (ISTA, 1993).

$$\text{Vigour Index} = \text{Percent germination} \times \text{seedling length (mean shoot length + mean root length} \quad \text{(Abdul-Baki and Anderson 1973)}$$

$$\text{Per cent Disease Incidence (PDI)} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

$$\text{Biocontrol Efficacy (BE)} = \frac{\text{DI of patho gentreated control} - \text{DI of antagonist (FLP) treatment}}{\text{Disease incidence of patho gentreated control}} \times 100$$

RESULT AND DISCUSSION

***Invitro* biocontrol efficiency of FLPs on major tomato phytopathogen:** Complete growth of FOL in the control plate was observed after 10 days of incubation. All the FLP isolates inhibited the growth of FOL *in-vitro.* Maximum percent of inhibition in the growth of FOL was recorded by FLP Brinjal New (37.64%), followed by FLP Barley (36.45%) and FLP Okra (36.85%), FLP Rice (33.40%) and FLP Chickpea (31.10%). *Alternaria solani* showed complete growth within 8 days of inoculation in the control plate at a temperature of 28°C. Maximum inhibition over control of the fungus was observed by FLP Rice (41.78%) followed by FLP Brinjal New (38.83%), FLP Barley (38.63%), FLP Okra (37.44%) and FLP Chickpea (29.39%). *Alternaria alternata* showed complete growth within 8 days of inoculation into the Petri plate at a temperature of 28°C. Maximum percent inhibition in the growth of the fungus was recorded by the FLP Brinjal New and FLP Barley at 37.64%, followed by the FLP Rice (37.45%), FLP Okra (37.44%), FLP Chickpea (27.36%). FLP Brinjal New that is a *P. flourescens* isolate, consistently performed better than the other isolates in dual culture against tomato pathogens (Table 1). This variability in ability to control different fungi, even when the fungal challenges are closely related has been document by earlier works. In a study done by, Ramyasmurthi *et al*. (2012), a singular *P. fluorescens* isolate was tested against *Aleternaria alternata, A. brassiceae, and A. brassicola* and the inhibition per cent presented was 16.5, 12.5 and 50 per cent respectively, here the same isolate showed variable inhibition of same fungal species. In another work by Hunziker *et al*. (2014), *Pseudomoans* spp. was tested against *Phytophthora infestans, Helminthosporium solani, Rhizoctonia solani, Fusarium oxysporum,* and *Dikeya dianthicola* with an inhibition percentage of 97, 50, 31, 2 and 44 per cent respectively, where the inhibition ranged from 2 to 97 per cent.

***Effect* of seed bacterization on germination and seedling growth in tomato:** Seed bacterization was evaluated using the paper towel method. The earliest germination was observed in FLP; Barley, Brinjal, and Okra bacterized seeds on 2nd day of incubation and on 3rd day of incubation for FLP Chickpea and FLP Rice bacterized seeds. The least number of days to 50 per cent seed germination was observed for FLP Brinjal (4days). The maximum germination percent was observed under FLP rice (96.33%) at par with Brinjal New (94.33%) followed by FLP Okra (92.33%), FLP barley (88.33%) and FLP chickpea (87.67%) as compared to untreated seed (83.00%). Tomato seeds (Var. Pusa Vishal) bacterized with FLP isolates were studied for their potential effects on seed germination and plant growth under *in vivo* pot culture experiments. Among the different treatments, maximum germination percent was recorded in FLP Brinjal New (95.33%) which was at par with FLP Barley (94.67%) followed by FLP Rice (92.67%) and FLP okra (90.60%). Least germination was recorded under untreated control and FLP rice (89.33%). Among, the different treatments maximum shoot length of 4.7 cm after 21 days of sowing was recorded under FLP Rice followed by the FLP Barley (3.83 cm), FLP Brinjal New, and Chickpea (3.8 cm). The minimum shoot length was recorded under FLP Okra (3.5cm) which was at par with untreated control. Similarly, maximum root length was observed under FLP Barley (3.83 cm) followed by FLP Brinjal New (3.67 cm), FLP Okra and Chickpea (3.50 cm), and minimum root length was seen in FLP Rice(1.83cm) which was at par with untreated control. The overall seedling length was maximum under FLP Rice (7.20 cm) followed by FLP Brinjal (6.20 cm) at par with the rest of the treatments. Maximum vigour index was recorded in FLP Brinjal New (940.26) at par with FLP Barley (912.26) and minimum vigour index was recorded in FLP Okra (810.6) at par with FLP Chickpea, FLP okra, and FLP chickpea. Whereas the lowest vigour index was recorded under untreated control (654.8 cm) (Table 2). Seed bacterization with PGPR is known to enhance germination percent shoot length, root length and seedling vigour by helping the plants through nutrient solubilization & assimilation, binding of iron through siderophores etc. (Masmoudi *et al*., 2019; Sharma *et al*., 2022).

Efficacy of seed bacterization on severity of early blight of tomato: The first disease incidence was observed four days post inoculation of the *Alternaria solani*. The maximum disease pressure of 23.76 percent was recorded under untreated control and minimum diseased index was found under FLP Barley (17.96%). The maximum percent reduction of disease over control was recorded under FLP barley (24.41) followed by FLP Okra (20.28), FLP Rice (19.57) and FLP Chickpea (12.92). The minimum percent reduction over control was of FLP Brinjal New (12.24). PGPR especially FLPs are known induce strong Induced Systemic Resistance (ISR) activity in different crops thereby enhancing plants resistance to foliar pathogens as well (Omoboye *et al.,* 2021; Dimkić *et al*., 2022).

Effect of seed bacterization on seedling health under tomato-fusarium wilt Pathosystem: The 1st seed germination was observed under treatments of FLP Barley. FLP Brinjal New germinated on 2nd day after sowing. On the 3rd day seed germination was observed across all treatments. The maximum germination percentage was recorded under FOL free control (91.4%). Among the pathogen challenged treatments maximum germination was recorded under FOL+FLP Barley (73.0%) at par with FOL+FLP Okra (72.20%) followed by the FOL+FLP Rice (71.40%), FOL+FLP Chickpea (70.20%) and FOL + FLP Brinjal (69.40). Least germination was recorded under FOL challenged non-bacterized seed treatment + FOL (64.20%). Significant improvements in shoot length of different treatments as compared to non-bacterized seed as well as pathogen control were observed. Maximum shoot length was found in FOL+ FLP Rice (4.42cm) followed by FOL+FLP Barley (4.00cm) and FOL+ FLP Chickpea (3.74cm) was recorded. The shoot length FOL+FLP Okra and FLP Brinjal New was found similar (3.60cm). The lowest shoot length was observed under pathogen control FOL (3.08cm). Similarly, maximum root length was recorded in FOL+FLP Rice (4.12cm) followed by the FOL+FLP Okra (3.94cm) and FOL+FLP Brinjal New (3.80cm) at par with FLP+FOL Barley. The minimum root length was recorded in FOL (3.20cm). Then seedling length was maximum under FOL+FLP Rice (8.54cm) followed by FOL+FLP Barley (7.66cm) at par with FOL+ FLP Okra (7.54cm). Minimum seedling length was observed in FOL (6.10cm). Maximum vigour index was recorded in FLP Rice (610.90) followed by FLP Barley (559. 50), FLP okra (544.22), FLP Chickpea (517.98), and FLP Brinjal new (513.44). Whereas FOL (391.66) recorded the lowest vigor index and control was at (654.54). The first signs of *Fusarium oxysporium* wilting symptoms was observed in pathogen control (FOL) where the typical wilting symptom like drooping of leaves followed by drying leading to complete wilting was observed after 10 days of germination (Table 3). The percentage of healthy plants ranged from 76 to 81.95 per cent among the treatments. The Maximum Disease incidence was recorded under control (34.25%). Among the FLP treatments, maximum reduction of disease over control was recorded under FOL+FLP Rice (44.71%) at par with FOL+FLP Okra (41.19%) followed by FOL+FLP Barley (32.84) and FOL+FLP Brinjal New (23.91%). Whereas FLP Chickpea was the least effective (20.62%). The seed bacterization with PGPR may lead to elicitation of defense related activities leading to activation of ISR activities. Numerous studies have shown the efficacy of FLPs in control of soil and foliar pathogens (Kaur *et al*., 2016; Singh *et al*., 2016).

Table 1*: In vitro* evaluation of Fluorescent Pseudomonad isolates against FOL and *Alternaria solani.*

Sr. No.	Isolate	Radial growth of FOL (mm)	Inhibition over control (%)	Radial growth of *A. solani* (mm)	Inhibition over control (%)
1.	FLP Barley	53.67	36.45^{a}	50.00	38.63^{ab}
2.	FLP Okra	53.00	36.85^{a}	52.00	37.44^{b}
3.	FLP Chickpea	62.33	31.10^{b}	65.00	29.39^{c}
4.	FLP Rice	58.67	33.40^{ab}	44.67	41.78^{a}
5.	FLP Brinjal N	51.67	37.64^{a}	49.67	38.83^{ab}
6.	Control	90	0.0	90	0.0

Table 2: Effect of seed bacterization of fluorescent pseudomonad isolates on seed germination and seedling vigour under tomato.

Sr. No.	Isolate	Germination (%)	Shoot length (cm)	Root length (cm)	Seedling length (cm)	Vigour Index	Disease Index (*A. solani*)	Per cent reduction over control
1.	FLP Barley	94.67^{a}	3.83^{b}	3.80^{a}	5.83^{b}	912.26^{a}	17.96^{a}	24.41
2.	FLP Okra	90.6^{ab}	3.5^{b}	3.50^{abc}	5.43^{b}	810.66^{ab}	18.94^{ab}	20.28
3.	FLP Chickpea	89.33^{b}	3.8^{b}	3.50^{abc}	5.83^{b}	834.66^{ab}	20.69^{ab}	12.92
4.	FLP Rice	92.67^{ab}	4.7^{a}	1.83^{c}	7.20^{a}	836.66^{ab}	19.11^{b}	19.57
5.	FLP Brinjal New	95.33^{a}	3.8^{b}	3.67^{ab}	6.20^{b}	940.26^{a}	20.85^{b}	12.24
6.	Control	89.33^{b}	3.8^{b}	1.97^{bc}	5.36^{b}	654.8^{a}	23.76^{b}	0

Table 3: Effect of seed bacterization of fluorescent pseudomonad isolates on seed germination and seedling vigour under tomato – *Fusarium oxysporum* f. sp. *lycopercici* Pathosystem.

Sr. No.	Isolate (+ FOL)	Germination (%)	Shoot length (cm)	Root length (cm)	Seedling length (cm)	Vigour Index	Disease Index (Wilt incidence)	Per cent reduction over control
1.	FLP Barley	73.0^{b}	4.00^{ab}	3.60^{ab}	7.66^{a}	559.50^{ab}	23.00^{bc}	32.84
2.	FLP Okra	72.2^{b}	3.60^{ab}	3.94^{ab}	7.54^{a}	544.22^{ab}	20.16^{c}	41.19
3.	FLP Chickpea	70.2^{bc}	3.74^{ab}	3.64^{ab}	7.38^{ab}	517.98^{b}	27.21^{b}	20.62
4.	FLP Rice	71.4^{b}	4.42^{a}	4.12^{a}	8.54^{a}	610.90^{ab}	18.95^{c}	44.71
5.	FLP Brinjal N	69.4^{bc}	3.60^{ab}	3.80^{ab}	7.40^{ab}	513.44^{b}	23.91^{bc}	30.25
6.	FOL	64.2^{c}	3.08^{b}	3.02^{b}	6.10^{b}	391.66^{c}	34.28^{a}	0.00
7.	Control	91.4^{a}	3.08^{b}	3.44^{ab}	7.16^{ab}	654.54^{a}	0.00^{d}	0.00

CONCLUSIONS

The research highlights the potential of native Fluorescent Pseudomonads (FLPs) isolated from diverse crops in Bihar for effective biocontrol of tomato pathogens, *Fusarium oxysporum* f. sp. *lycopersici* and *Alternaria* spp. The study demonstrates the positive impact of FLP seed bacterization on germination, seedling vigor, and disease resistance. These findings underscore the significance of harnessing native microbial strains as a sustainable and promising strategy for managing pests and diseases in high-value vegetable crops like tomato, contributing to improved food safety and agricultural sustainability.

FUTURE SCOPE

Further investigation could focus on optimizing the application methods of these FLPs for practical field implementation, assessing their long-term effects on soil health, and exploring their potential in integrated pest management strategies.

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Conflict of Interest. None.

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