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Biological Control of Early Blight of Tomato (*Solanum lycopersicum* L.) by the Use of *Pseudomonas fluorescens* (Flugge and Migula) under Field Condition

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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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Short Research Article

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ABSTRACT

Tomato is the second most important vegetable crop. It is widely grown on all over the world. Diseases are the major constraint in economic crop production as they inflict heavy loss in tomato. Among the various fungal diseases, early blight was reported severe as it causes heavy damage to the crop. The main aim of this study is to explore the potentiality of native and most efficient isolate

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of *P. fluorescens* as biocontrol agent. A total 30 *Pseudomonas fluorescens* isolates were isolated from soil samples of different villages of Navsari district by serial dilution method on King's B medium. The isolates were purified by observing under UV light. A field experiment was conducted for evaluation of *P. fluorescens* against diseases of tomato. Among the different treatments of *P. fluorescens*, treatment T8 i.e. combined use of all treatments recorded with minimum per cent disease intensity (37.78%) against early blight disease and maximum per cent disease intensity (69.33%) was recorded in the control (T9, water spray).

Keywords: Biological control; P. fluorescens; tomato; early blight.

1. INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is the second most important vegetable crop. It is one of the most important vegetable crops in the world as well as in India considered as "Protective Food" both because of its special nutritive value. The nutritive value of tomato per 100g of edible portion are as moisture 93.19 per cent, protein 1.90 g, potassium 144mg, copper 0.19mg, sulphur 24 mg, chlorine 38.00 mg, vitamin C 31.00 mg, thiamine 0.07 mg, riboflavin 0.01 mg. nicotinic acid 0.40 mg, magnesium 15.00mg, oxalic acid 2.00 mg, phosphorous 36.00 mg, Iron 1.80 mg and vitamin A 320.00 mg [1]. More than 200 diseases have been reported to infect tomato in the world. Several of fungal diseases such as early blight (*Alternaria solani* Ellis and Martin), late blight (*Phytophthora infestans* De Bary), Septoria leaf blight (*Septoria lycopersici* Speg.), powdery mildew (*Oidiopsis taurica*), Fusarium wilt (*Fusarium oxysporum* f. sp. *lycopersici* Snyder and Hansen), collar rot (*Sclerotium rolfsii* Sacc.), and damping-off (*Pythium aphanidermatum*) are causes severe losses in tomato. Among the fungal diseases, early blight (*A. solani*) is one of the most important and frequent occurring disease nationally and worldwide in the crop [2].

Pseudomonas fluorescens representing group of PGPR can promote growth and suppress plant pathogens by multiple mechanisms. Their applicability as biocontrol agents has drawn wide attention because of production of secondary metabolites such as siderophore, antibiotics, volatile compounds, HCN, enzymes and phytohormones [3]. They can be utilized in low input sustainable agricultural applications, such as biocontrol, on account of their ability to synthesize secondary metabolites with antibiotic properties and many of such antibiotics produced have a broad spectrum activity but strain to strain variations do exist [4].

2. MATERIALS AND METHODS

2.1 Soil Sample Collection and Isolation of *P. fluorescens*

Soil sampling was carried out from different rhizospheric soils of tomato, chilli and brinjal field from Navsari and adjoining area. Rhizospheric soil samples of Materials and methods 23 selected crop plants up to a depth of 10 to 15cm. The soil intimately adhering to the roots was collected and mixed to provide a composite soil sample. The GPS data (Latitude ºN and Longitude ºE) was collected at each sampling site distributed over the entire Navsari district of Gujarat state (Table 1). Ten gram of soil from each sample was taken in a conical flask to which 90 ml of normal saline water was added. The sample was agitated for 15 min., on a vortex and serial dilutions of soil suspensions were prepared. Serial dilutions prepared for the rhizobacteria. For *P. fluorescens* 10^{-2} to 10^{-5} dilutions were taken and 0.1 ml of respective dilutions were spread on sterilized Petri plates containing specific media i.e., King's B (*P. fluorescens*) and the Petri plates were incubated at room temperatures (28º±2ºC) for 24-72hrs. Three repetitions were maintained for each dilution. The plates were examined daily up to three days for bacterial colonies. Pure cultures of isolated colonies were obtained by the streak plate method [5].

2.2 Efficacy of Efficient *P. fluorescens* Isolate against Early Blight of Tomato in Field

During a semi-rabi season in 2019-20, field experiment was carried out at NAU's college farm in Navsari. In the present study, different treatments and combinations of treatments, as described in Table 2 were evaluated to determine the efficacy of an efficient *P. flurorescens* isolate against early blight disease in tomato. *P. flurorescens* were applied by

different method *viz.* seed treatment 1 kg seeds were soaked in *P. fluorescens* suspension (10 ml) for 10min.), in a seedling dip method seedlings were dipped in the *P. fluorescens* formulation @ 10ml/lit. of water (1×10^8 cfu/ml) for 30 min., and the seedlings were transplanted). The first spray was applied at the start of the disease, and subsequent sprays were applied 20 and 40 days later in case of spraying treatment.

Observations were recorded by using following 0-5 scale [6].

Table 1. Details of the soil sampling

Sr. No.	Latitude ºN	Longitude ºE	Village	Taluka	Crop	Stage of crop
1	20º55'19"	72º52'57"	Pethan	Jalalpore	Brinjal	Fruiting
2	20º54'37"	72º55'06"	Hansapore	Jalalpore	Brinjal	Fruiting
3	20º54'52"	72º51'51"	Kothmadi	Jalalpore	Tomato	Fruiting
4	20º52'27"	73º03'46"	Matwd	Jalalpore	Chilli	Fruiting
5	20º55'39"	72º52'36"	Karadi	Jalalpore	Brinjal	Fruiting
6	20º53'14"	72º55'10"	Mandir	Jalalpore	Chilli	Fruiting
7	20º53'12"	72º55'13"	Mandir	Jalapore	Brinjal	Fruiting
8	20º53'40"	72º55'03"	Mandir	Jalapore	Tomato	Fruiting
9	20º49'28"	73º18'52"	Palgabhan	Vansda	Brinjal	Fruiting
10	20º49'13"	73º19'23"	Palgabhan	Vansda	Chilli	Fruiting
11	20º49'31"	73º19'45"	Bhinar	Vansda	Brinjal	Fruiting
12	20º49'02"	73º19'44"	Bhinar	Vansda	Tomato	Fruiting
13	20º51'27"	72º59'33"	Gadat	Gandevi	Brinjal	Fruiting
14	20º45'56"	73º03'02"	Samroli	Chikhali	Tomato	Fruiting
15	20º46'12"	73º02'42"	Samroli	Chikhali	Brinjal	Fruiting
16	20º51'22"	73º18'58"	Sindhai	Vansda	Brinjal	Fruiting
17	20º51'28"	73º18'55"	Sindhai	Vansda	Tomato	Fruiting
18	20º51'10"	73º18'58"	Unai	Vansda	Brinjal	Fruiting
19	20º50'52"	73º18'55"	Unai	Vansda	Tomato	Fruiting
20	20º54'03"	72º58'22"	Adada	Navsari	Chilli	Fruiting
21	20º54'26"	72º58'31"	Adada	Navsari	Brinjal	Fruiting
22	20º48'34"	73º09'17"	Rankuva	Chikhli	Brinjal	Fruiting
23	20º48'26"	73º08'36"	Rankuva	Chikhli	Tomato	Fruiting
24	20º48'45"	73º11'31"	Kukeri	Chikhli	Tomato	Fruiting
25	20º48'40"	73º11'20"	Kukeri	Chikhli	Brinjal	Fruiting
26	20º51'11"	72º59'03"	Sonvadi	Gandevi	Brinjal	Fruiting
27	20º49'29"	72º59'44"	Ajrai	Gandevi	Chilli	Fruiting
28	20º50'34"	72º59'30"	Khakhwada	Gandevi	Tomato	Fruiting
29	20º55'23"	72º53'25"	NAU Farm	Jalalpore	Tomato	Fruiting
30	20º55'23"	72º53'27"	NAU Farm	Jalalpore	Brinjal	Fruiting

Table 2. Treatments given to the tomato

No.	Treatment
T_1	Removal of infected leaves, staking of plants
T_2	Seed treatment of *P. fluorescens* @ 10 ml/kg seed (1×10^9 cfu/ml)
T_3	Seedling dip treatment of *P. fluorescens* @ 10 ml/lit of water (1×10^9 cfu/ml)
T_4	Furrow application of *P. fluorescens* @ 0.1 % mixed in well decomposed FYM (1×10^9cfu/ml)
T_5	Foliar spray of *P. fluorescens* @ 6 ml/lit of water (1×10^9 cfu/ml) (Two time application: First spray at initiation of disease and second at 20 days after first application)
T_6	Foliar spray of *P. fluorescens* @ 6 ml/lit of water (1×10^9 cfu/ml) (Three time application:First spray at initiation of disease and subsequent at 20 and 40 days after first application, respectively)
T_7	Combination of treatments T_1 to T_5
T_8	Combination of treatments T_1 to T_6
T_9	Control (Water spray)

Table 3. Disease rating scales for early blight disease

Scale	Description of the symptoms
0	Leaves free from infection
1	Small irregular spots covering <5 per cent leaf area
2	Small irregular brown spots with concentric rings covering 5.1-10 per cent leaf area
3	Lesion enlarging irregular brown with concentric rings covering 10.1-25 per cent leaf area
4	Lesions coalesce to form irregular and appears as a typical blight symptom covering 25.1- 50 per cent leaf area
5	Lesions coalesce to form irregular and appears as a typical blight symptom covering > 50 per cent leaf area

Per cent Disease Intensity (PDI) was calculated by using following formula of Wheeler [7] as given here:

$$\text{Per cent disease intensity (PDI)} = \frac{\text{Sum of all ratings of plants observed}}{\text{Total number of plantsexamined} \times \text{maximum rating}} \times 100$$

3. RESULTS AND DISCUSSION

3.1 Soil Sample Collection and Isolation of *P. fluorescens*

Total 30 isolates were obtained from 30 different soil samples by serial dilution method on King's B Agar medium. *Pseudomonas fluorescens* colonies were selected by observing under UV light.

3.2 Efficacy of Efficient *P. fluorescens* Isolate against Early Blight of Tomato in Field

Effect of different treatments of *P. fluorescens* was recorded for the management of early blight disease of tomato. Per cent disease intensity (PDI) was calculated by use of 0-5 scale based on the symptoms showed on leaf (Fig. 1). The data of early blight PDI was recorded for five times at 45, 60, 75, 90 and 105 days after transplanting. At the time of transplanting (30 days old seedling) none of the seedlings showed any disease symptoms. All the treated plots with *P. fluorescens* recorded significantly less per cent disease intensity over control at 105 days after transplanting. Maximum per cent disease intensity (69.33%) was recorded in the control (T9, water spray). Among different treatments, treatment T8 (combination of T1 to T6 treatments) was found most effective on early blight with 37.78 per cent, followed by treatment T7 (39.55%, combination of T1 to T5 treatments), T2 (43.11%, seed treatment of *P. fluorescens*), T3 (45.33%, seedling treatment of *P. fluorescens*), T4 (51.55%, furrow application of *P. fluorescens*) treatments. Least control of the disease was recorded in T1 treatment (60.44%, removal of infected leaves, Staking), followed by treatment T5 (57.33% two time spray of *P. fluorescens*), T6 (55.55%, three time spray of *P. fluorescens*) and treatment T10 (control) recorded 65.33% PDI (Table 4 and Fig. 2).

The data also showed that among all the treatments, there was increase in disease intensity from 45 to 105 days after transplanting. However, the rate of increase in per cent disease intensity was slow in case of treated plots compared to the control plots. Among the all treatment T8 treatment (combination of T1 to T6 treatments) were found highly effective to management early blight of tomato. The result in terms of per cent disease over control presented in Table 5 and Fig. 3, Revealed that highest per cent disease control (42.17%) recorded in T8 (combination of T1 to T6) treated plot followed by T7 (39.46%, combination of T1 to T5) treated plot, T2 (34.01%, seed treatment of *P. fluorescens*), T3 (30.61%, seedling dip of *P. fluorescens*), T4 (21.09%, furrow application of *P. fluorescens*), T6 (14.97%, three time spray of *P. fluorescens*), T5 (12.24%, two time spray of *P. fluorescens*) and T1 treatment (7.59 %, removal of infected leaves, staking).

Table 4. Effect of different treatments *P. fluorescens* on PDI (Per cent disease intensity) of early blight in tomato at different intervals

No.	Treatment	PDI				
		45 DAT	**60 DAT**	**75 DAT**	**90DAT**	**105DAT**
T_1	Removal of infected leaves, staking of plants	25.88* (19.11)	32.19 (28.44)	37.12 (36.44)	44.36 (48.89)	51.04 (60.44)
T_2	Seed treatment of *P. fluorescens*	19.04 (10.66)	23.20 (15.55)	27.77 (21.77)	33.85 (31.11)	41.00 (43.11)
T_3	Seedling dip treatment of *P. fluorescens*	20.65 (12.44)	25.90 (19.11)	29.01 (23.55)	35.77 (34.22)	42.31 (45.33)
T_4	Furrow application of *P. fluorescens* by mixed inwell decomposed FYM	23.20 (15.55)	28.10 (22.22)	31.92 (28.00)	39.47 (40.44)	45.90 (51.55)
T_5	Foliar spray of *P. fluorescens* (two time application)	26.86 (20.44)	31.07 (26.66)	34.97 (32.89)	42.32 (45.33)	49.22 (57.33)
T_6	Foliar spray of *P. fluorescens* (three time application)	26.22 (19.55)	31.57 (27.44)	34.50 (32.11)	41.80 (44.44)	48.20 (55.55)
T_7	Combination of treatments T_1 to T_5	14.76 (6.66)	20.25 (12.00)	24.91 (17.78)	31.62 (27.55)	38.96 (39.55)
T_8	Combination of treatments T_1 to T_6	15.25 (7.11)	19.42 (11.11)	23.38 (16.00)	30.48 (25.77)	37.89 (37.78)
T_9	Control (Water spray)	32.79 (29.33)	37.13 (36.44)	42.32 (45.33)	48.70 (56.44)	56.41 (69.33)
T_{10}	Absolute Control (No spray)	32.22 (28.44)	36.31 (35.11)	41.55 (44.00)	47.17 (53.77)	53.98 (65.33)
SEm ±		1.14	1.09	1.33	1.44	1.80
CD at 5%		3.37	3.24	3.96	4.27	5.35
CV (%)		8.30	6.62	7.05	6.29	6.70

** Figure outside parentheses indicated arsine transformed values*

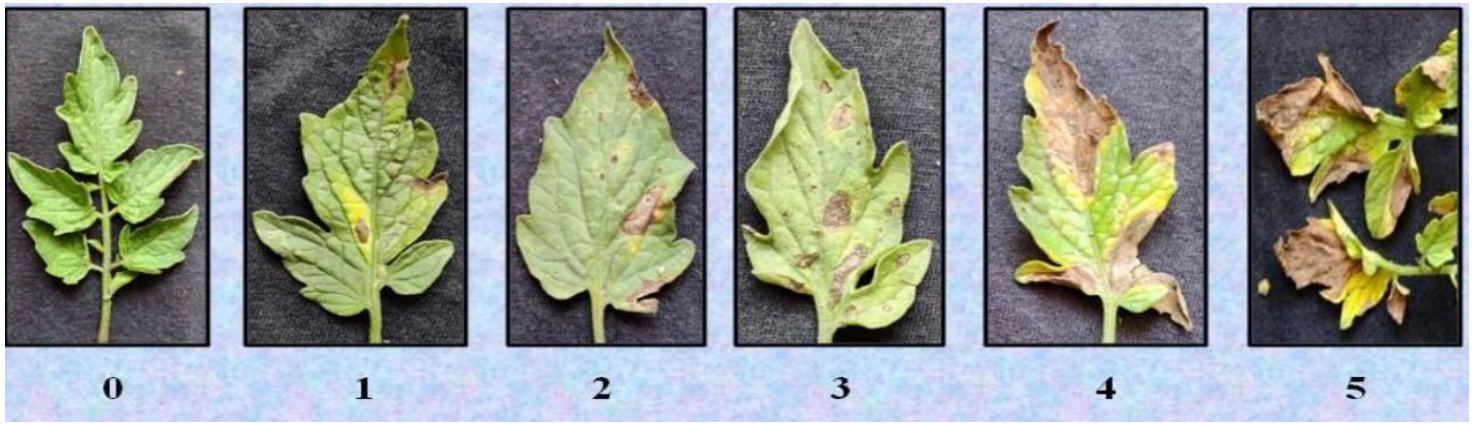

Fig. 1. Disease scoring scale for early blight of tomato (0-5 scale)

Table 5. Effect of different treatments of *P. fluorescens* for diseases control of early blight intomato under field conditions

No.	Treatments	Early blightdisease intensity	Disease Control (%)
T_1	Removal of infected leaves, staking of plants	51.04* (60.44)	7.59
T_2	Seed treatment of *P. fluorescens*	41.00 (43.11)	34.01
T_3	Seedling dip treatment of *P. fluorescens*	42.31 (45.33)	30.61
T_4	Furrow application of *P. fluorescens* by mixed in welldecomposed FYM	45.90 (51.55)	21.09
T_5	Foliar spray of *P. fluorescens* (two time application)	49.22 (57.33)	12.24
T_6	Foliar spray of *P. fluorescens* (three time application)	48.20 (55.55)	14.97
T_7	Combination of treatments T_1 to T_5	38.96 (39.55)	39.46
T_8	Combination of treatments T_1 to T_6	37.89 (37.78)	42.17
T_9	Control (Water spray)	56.41 (69.33)	-
T_{10}	Absolute Control (No spray)	53.98 (65.33)	-
SEm ±		1.80	-
CD at 5%		5.35	-
CV (%)		6.70	-

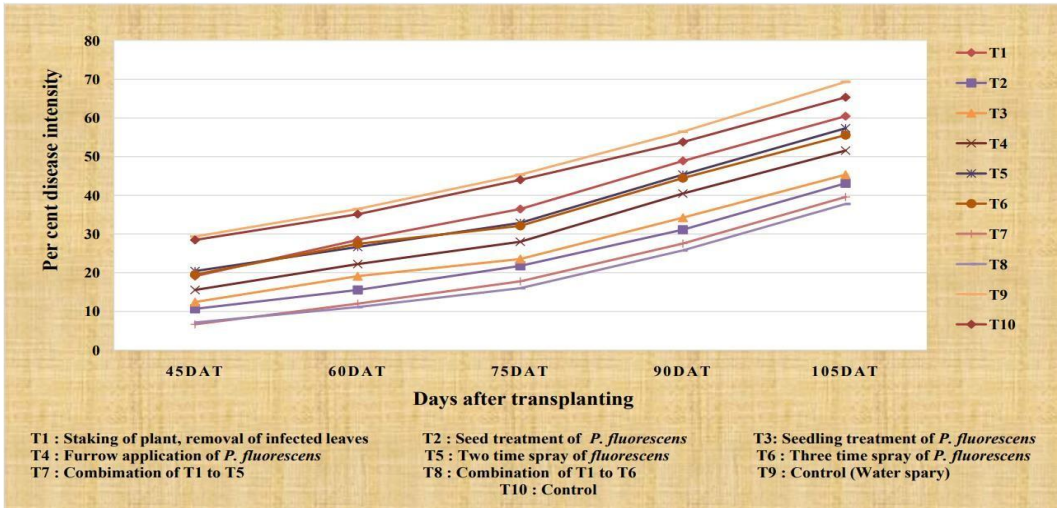

Fig. 2. Effect of different treatments of *P. fluorescens* on per cent disease intensity early blight intomato at different intervals

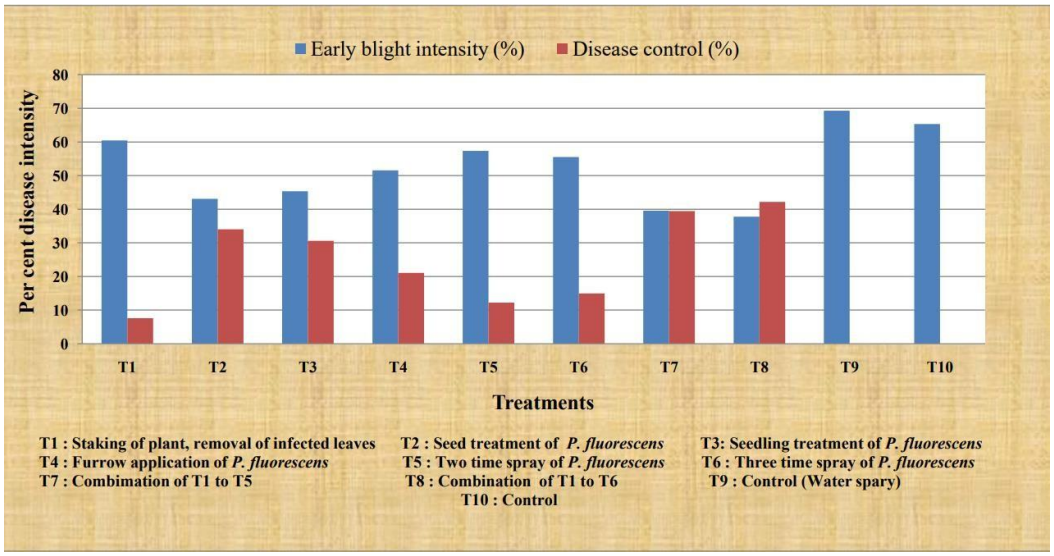

Fig. 3. Effect of different treatments of *P. fluorescens* for disease control of tomato early blight inField conditions

Verma et al. [8] evaluated the efficacy of biocontrol agents and botanicals against early blight of tomato caused by *A. solani* in net house. They reported that P. *fluorescens* was effective in reducing disease severity (25.33%) compared to untreated control (40.33%). Dhal et al. [9] conducted field experiment to study the synergistic effect of cultural practices, seed priming and foliar spray of bioagents for management of early blight of tomato. The foliar spray of *P. fluorescens* with priming of seeds proved effective by reduced the disease 73.00 per cent as well as increasing the yield 43.70 per cent, respectively.

4. CONCLUSION

P. fluorescens is act as an effective biocontrol agent against early blight of tomato under field condition. Different method of applications of *P. fluorescens* was used for the management of early blight disease among them, T_8 i.e. combined use of all treatments were found most effective with minimum per cent disease intensity and maximum disease control followed by T_7i.e. combined application of all treatments except T_6 i.e. foliar spray of *P. fluorescens* (three time application).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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