Biological control of early blight of tomato (*Solanum lycopersicum* L.) by the use of *Pseudomonas fluorescens* (Flugge and Migula) under field condition

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. A total 30 *P. 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 (combination of T1 to T6) recorded minimum per cent disease intensity (37.78%) against early blight disease.

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Keywords: Biological control, *P. fluorescens*, tomato, early blight

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 1.90g, 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 2.00mg, phosphorous 36.00 mg, Iron 1.80 mg and vitamin A 320.00 mg (Verma *et al*. 2018). 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*) one most important and frequent occurring disease of the crop nation and worldwide.

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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 (Gupta *et al.* 2001). 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 (Raaijimakers *et al.* 2002).

Material and Methods

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 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. 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 (Vlassak *et al.* 1992).

Comment [S4]: you should complete a map of the research location

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 1, 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 (10ml) for 10min.), in a seedling dip method Seedlings were dipped in the *P. fluorescens* formulation @ 10ml/lit. of water (1×108 cfu/ml) for 30min., and the seedlings were transplanted) and The first spray was applied at the start of the disease, and subsequent sprays were applied 20 and 40 days later.

Table 1: 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)

Observations were recorded using a 0-5 scale (Horsfall and Barratt., 1945).

Table 2: Disease rating scales for early blight disease:

Comment [S5]: do not cut the table

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 (1969) as given here:

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

Results and discussion

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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.

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.

Comment [S7]: preferably complete with a table

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 (Table 3 and Fig. 4.). 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 fig. 2. 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) (Fig. 3).

Comment [S8]: preferably complete with a table

Verma *et al.* (2018) 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.* (2017) 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.

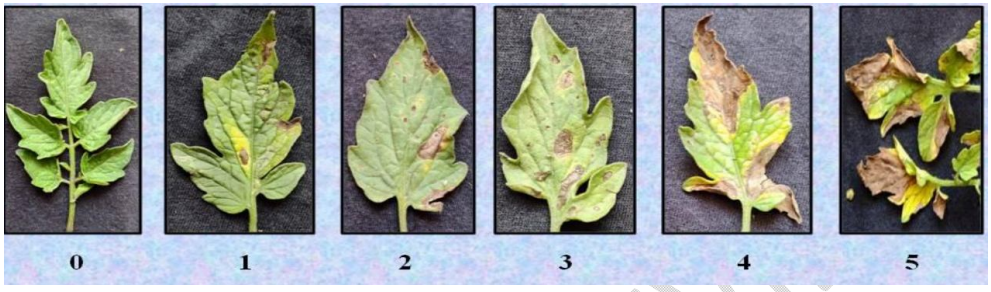

Fig. 1 Disease scoring scale for early blight of tomato (0-5 scale)

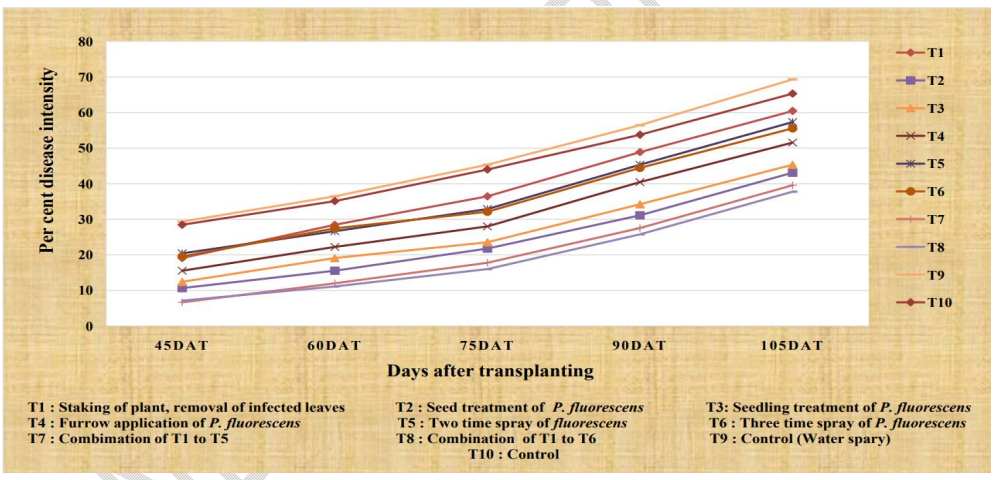

Fig. 2 Effect of different treatments of *P. fluorescens* on per cent disease intensity early blight in tomato at different intervals

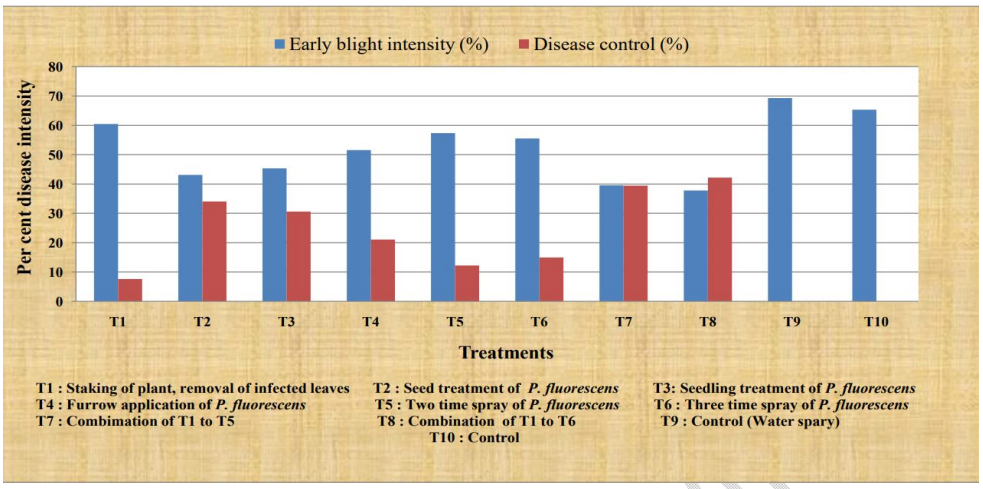

Fig. 3 Effect of different treatments of *P. fluorescens* for disease control of tomato early blight in Field conditions

Reference

Dhal, A., Beura, S. K., Dash, S. K., Tripathy, L., Swain, S. K. and Sethi, D. (2017). Eco-friendly and integrated approaches for management of early blight disease in tomato. *International Journal of Current Microbiology and Applied Sciences*, **6** (10): 3052-305.

Gupta, C. P., Dubey, R. C., Kang, S. C. and Maheshwari, D. K. (2001). Antibiosis-mediated necrotrophic effect of Pseudomonas GRC2 against two fungal plant pathogens. Current Science, **81**: 91-94.

*Horsfall, J. G. and Barratt, R. W. (1945). An improved system for measuring plant diseases. *Phytopathology*, **35**: 655. (FIDE: http://en.wikipidia.org./w/index.php?title=Horsfall Barratt_scale&oldid=3 78866242)

Raaijmakers, J. M., Vlami, M. and de Souza, J. T. (2002). Antibiotic production by bacterial biocontrol agents. *Antonie Van Leeuwenhoek*, **81**: 537–547.

Verma, A., Kumar, S., Harshita, Shina, A., and Jaiswal, S. (2018). Evaluate the efficacy of bio-control agent and botanicals against early blight of tomato caused by *Alternaria solani*. *The Pharma Inovation Journal*, **7** (3):28-30.

Verma, A., Kumar, S., Harshita, Shina, A., and Jaiswal, S. (2018). Evaluate the efficacy of bio-control agent and botanicals against early blight of tomato caused by *Alternaria solani*. *The Pharma Inovation Journal*, **7** (3):28-30.

Vlassak, K. L., Van, H. and Duchateau, L. (1992). Isolation and characterization of *fluorescent Pseudomonas* associated with the roots of rice and banana grown in Srilanka. *Plant and Soil*, **145**: 51-63.

Wheeler, B. E. J. (1969). An Introduction to Plant Diseases. John Wiley and Sons Limited, London. pp. 301.