

Original Research Article

<https://doi.org/10.20546/ijcmas.2020.907.182>

Invitro efficacy of *Pseudomonas fluorescens* and *Bacillus subtilis* against *Fusarium oxysporum* f. sp. *lycopersici*

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ABSTRACT

Keywords

Tomato (*Solanum lycopersicum* L.),
Pseudomonas fluorescens,
Bacillus subtilis
Fusarium oxysporum

Article Info

Accepted:
14 June 2020
Available Online:
10 July 2020

Tomato (*Solanum lycopersicum* L.) is one of the most widely grown vegetable crops in the world. 'Lycopene' produced only by tomato is a natural antioxidant that works effectively to slow the growth of the cancerous cells. Tomato plant is susceptible to various diseases caused by different agents such as Bacteria, Viruses, Nematode, Fungi and Abiotic factors. Among the fungal diseases, wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* causes economic loss of tomato production worldwide. PGPR playing a vital role and capable to colonize the plants root system and improve the growth and yield. In this present study biocontrol agents viz, *Bacillus subtilis* and *Pseudomonas fluorescens* have been tested against the tomato wilt pathogen *Fusarium oxysporum* f. sp. *lycopersici*. In the present study the from among the *Pseudomonas* isolates collected, the isolate Pf₅ collected from Puthur showed the maximum inhibition and significantly inhibited the growth of *F. oxysporum* f. sp. *lycopersici* (37.12mm), which was 58.75 per cent reduction on the growth of the pathogen. In the poisson food technique the maximum reduction in the growth of mycelium is noticed in the isolate Pf₅ with 45.89 mm, 28.45mm, 16.78mm and 8.46mm 10, 20, 30 and 40 percent respectively. And among the *Bacillus* isolate collected the isolate Bs₆ collected from Arasur showed the maximum inhibition and significantly inhibited the growth of *F. oxysporum* f. sp. *lycopersici* (32.63 mm), which was 63.74 per cent reduction on the growth of the pathogen when compared to control. In poison food technique the maximum reduction in the growth of mycelium is noticed in the isolate Bs₆ with 40.48mm, 31.23mm, 15.78mm and 3.23mm at 10, 20, 30 and 40 percent respectively.

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most widely grown vegetable crops in the world (Pastor *et al.*, 2012). Tomato is used for consumption due to its high nutritive values, antioxidant and curative properties (Sahu *et al.*, 2013). Tomatoes are excellent source of

various micronutrients and antioxidants. It has high nutritional values which plays a crucial role in our daily home cooking (Prachi singh *et al.*, 2019). Tomato contains high value of vitamin C, Lycopene, and β -Carotene, which supports and promote good health. The nutritional quality of tomato is mainly determined by its Carotenoid, Potassium,

Vitamin C and vitamin A content. Ripe tomatoes have high levels of Carotenoids, of which carotenes make up between 90 and 95% (Guil-Guerrero and Reboloso-Fuentes, 2009). 'Lycopene' produced only by tomato is a natural antioxidant that works effectively to slow the growth of the cancerous cells (Bhovomik *et al.*, 2012).

Around the globe China ranks first in the world with an area of 14.5 lakh hectares and 41.626 million tonnes of production per annum (Anon., 2015). India occupies second position in the world with respect to area, but occupies only fifth place in terms of production. Total area under tomato cultivation in India is 7.97 lakh ha with a production of 207.08 lakh tonnes (Anonymous 2018). In Tamil Nadu the area under tomato cultivation is 25370 ha with the production of 328.2 tonnes per ha (Dhivya *et al.*, 2018). Tomato plant is susceptible to various diseases caused by different agents such as Bacteria, Viruses, Nematode, Fungi and Abiotic factors (Sahu *et al.*, 2013). Tomato production is hampered by soil borne pathogens such as *Fusarium* wilt and Bacterial wilt etc. Among the fungal diseases, wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* causes economic loss of tomato production worldwide.

Management of *Fusarium* wilt is mainly done by Chemical pesticides such as Pentachloro-nitrobenzene (PCNB) and soil fumigants as vapam, chloropicrin and methyl bromide. These agrochemicals are associated with several issues including phytotoxicity, pesticide residue, health hazards and environmental disaster (Stevens *et al* 2003). So the research was augmenting various bio-methods to freeze out the various issues.

PGPR playing a vital role and capable to colonize the plants root system and improve the growth and yield. Plant growth promoting

Rhizobacteria with biocontrol traits can be considered as an alternative to the high doses of pesticides applied on crops to deter the pathogens and reduce the disease severity ((Mahendra Prasad *et al.*, 2019). Many of the PGPR strains produce active metabolize that are inhibitory to pathogen and suppress their growth (Beneduzi *et al.*, 2012). Isolates of *Pseudomonas* spp. and *Bacillus* spp. recovered from tomato rhizosphere were positive for HCN production which is able to control *Fusarium* wilt of tomato caused by *Fusarium* sp. (Lachisa and Dabassa 2015). These bacteria have been broadly described for wide range antagonistic activities to combat phytopathogens. (Tariq *et al.*, 2010). *Bacillus subtilis* is also having significant antagonistic activity against *F. oxysporum* in both laboratory and *in vivo* conditions. The *B. subtilis* strain EU07 reduced the incidence of disease caused by *F. oxysporum* f. sp. *lycopersici* by 75% (Rocha, 2017). In this present study biocontrol agents viz, *Bacillus subtilis* and *Pseudomonas fluorescens* have been tested against the tomato wilt pathogen *Fusarium oxysporum* f. sp. *lycopersici*.

Materials and Methods

Dual culture (Dennis and Webster, 1971)

A nine mm culture disc obtained from the periphery of the seven days old culture of *F. oxysporum* f.sp. *lycopersici* was inoculated at 75mm approximately away from the edge of the Petri dish containing 15 ml of sterilized and solidified PDA medium. The bacterial antagonist *Pseudomonas* and *Bacillus* were streak gently made onto the medium using two days old culture just opposite to the pathogenic culture at equidistance. The zone of inhibition and the mycelial growth of *F. oxysporum* f. sp. *lycopersici* were recorded. The effective antagonists were selected based on the inhibition of the growth of the pathogen. The per cent inhibition of mycelial

growth was calculated according to Vincent (1927).

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

Where C – Radial growth (mm) in Control, T = Radial growth (mm) in Treatment

Bioassay of Culture filtrates of the antagonist on the mycelial growth of *Fusarium oxysporum* f. sp. *lycopersici*:

Effect of culture filtrates on the growth of *F. oxysporum* f. sp. *lycopersici* (Poison food technique)

The culture filtrates of the antagonists were separately incorporated into sterile PDA melted medium at 10, 20 and 30 percent concentrations by means of a sterile pipette. The amended media were transferred to sterile petri dishes separately @ 15 ml and allowed to solidify. The PDA medium without the culture filtrate served as control. Each plate was inoculated at the centre with seven days old pathogen culture. Three replications were maintained for each treatment. The diameter of the mycelial growth (mm) of the pathogen was measured after 7 days of incubation.

Results and Discussion

In vitro efficacy of *Pseudomonas fluorescens* against *Fusarium oxysporum* f. sp. *lycopersici* (Fol₃) (Dual culture)

In general all the native *Pseudomonas fluorescens* tested significantly inhibited the mycelial growth of *F. oxysporum* f. sp. *lycopersici* (Table 1). However, among the isolates, the isolate Pf₅ collected from Puthoor showed the maximum inhibition and significantly inhibited the growth of *F. oxysporum* f. sp. *lycopersici* (37.12mm),

which was 58.75 per cent reduction on the growth of the pathogen when compared to control. This was followed by the isolates Pf₆ and Pf₉ in the decreasing order of merit, which inhibited the growth of *F. oxysporum* f. sp. *lycopersici* by 52.36 and 49.37 per cent over control. The least growth inhibition of the pathogen (19.27 %) was exhibited by the isolate Pf₄.

Efficacy of *Pseudomonas fluorescens* against *Fusarium oxysporum* f. sp. *lycopersici* (Fol₃) (Poison food technique)

The results depicted in table 2 showed that the different isolates of *Pseudomonas fluorescens* significantly inhibited the growth of *F. oxysporum* f. sp. *lycopersici*. The maximum reduction in the growth of mycelium is noticed in the isolate Pf₅ with 45.89 mm, 28.45mm, 16.78mm and 8.46mm with percent inhibition of 49.01%, 68.38%, 81.35% and 90.60% at 10%, 20%, 30% and 40% respectively. And the minimum reduction in the growth of mycelium is noticed in the isolate Pf₄ with 61.26mm, 38.31mm, and 27.62mm and 16.46mm with percent inhibition of 31.93%, 57.43%, 69.31% and 81.71% at 10%, 20%, 30% and 40% respectively.

In vitro efficacy of *Bacillus subtilis* against *Fusarium oxysporum* f. sp. *lycopersici* (Fol₃) (Dual culture)

In general all the native *Bacillus* spp. tested significantly inhibited the mycelial growth of *F. oxysporum* f. sp. *lycopersici* (Table 3). However, among the isolates, the isolate Bs₆ collected from Arasur showed the maximum inhibition and significantly inhibited the growth of *F. oxysporum* f. sp. *lycopersici* (32.63 mm), which was 63.74 per cent reduction on the growth of the pathogen when compared to control. The least growth inhibition of the pathogen (24.18 %) was exhibited by the isolate Bs₄.

Efficacy of *Bacillus subtilis* against *Fusarium oxysporum* f. sp. *lycopersici* (Fol₃) (Poison food technique)

The results depicted in table 4 showed that the different isolates of *Bacillus subtilis* significantly inhibited the growth of *F. oxysporum* f. sp. *Lycopersici*. The maximum reduction in the growth of mycelium is noticed in the isolate Bs₆ with 40.48mm, 31.23mm, 15.78mm and 3.23mm with percent inhibition of 55.02 %, 65.30%, 82.46% and 96.41% at 10%, 20%, 30% and 40% respectively. And the minimum reduction in the growth of mycelium is noticed in the isolate Bs₃ with 56.35mm, 46.12mm, and 30.61mm and 18.46mm with percent inhibition of 37.38%, 48.47%, 65.98% and 79.48% respectively.

In vitro efficacy of *Pseudomonas fluorescens* against *Fusarium oxysporum* f. sp. *lycopersici*

Among the isolates, the isolate Pf₅ collected from Puthur showed the maximum inhibition

and significantly inhibited the growth of *F. oxysporum* f. sp. *lycopersici* (37.12mm), which was 58.75 per cent reduction on the growth of the pathogen. In the poison food technique the maximum reduction in the growth of mycelium is noticed in the isolate Pf₅ with 45.89 mm, 28.45mm, 16.78mm and 8.46mm with percent inhibition of 49.01%, 68.38%, 81.35% and 90.60% at 10%, 20%, 30% and 40% respectively. There are various modes of actions such as antibiosis, competition for iron through production of siderophores, parasitism that may involve production of extracellular enzymes and induction of plant resistance mechanisms (Naureen *et al.*, 2015). Several earlier workers have suggested that the inhibitory action might be due to production of an antimicrobial arsenal, including hydrogen cyanide (HCN), antibiotics, pyoluteorin, phenazines, pyrrolnitrin, siderophores, cyclic lipopeptides, and 2,4-diacetylphloroglucinol (DAPG), phytohormones, solubilisation of phosphate as well as excrete hydrolytic enzymes, such as protease, cellulase, chitinase, β -1,3 glucanase (Kumar *et al.*, 2007).

Table.1 In vitro efficacy of *Pseudomonas fluorescens* against *Fusarium oxysporum* f. sp. *lycopersici* (Fol₃) (Dual culture)

S. No	Isolates	Locality	Mycelial growth (mm)	Percent inhibition over control (%)
1.	Pf ₁	Hamumantheertham	52.46 ^d	41.71
2.	Pf ₂	Irumathur	64.23 ^g	28.63
3.	Pf ₃	Uthangarai	69.36 ⁱ	22.93
4.	Pf ₄	Thippampatti	72.65 ^h	19.27
5.	Pf ₅	Puthoor	37.12 ^a	58.75
6.	Pf ₆	Arasur	42.87 ^b	52.36
7.	Pf ₇	Kollanaikanoor	56.78 ^e	36.91
8.	Pf ₈	Mittapalli	47.58 ^c	47.13
9.	Pf ₉	Kodamandapatti	45.56 ^d	49.37
10.	Pf ₁₀	Mathur	60.87 ^f	32.36
11.	Control	-	90.00	-

* Mean of three replications; * In a column, means followed by a common letter are not significantly differ at 5% level by Duncan’s multiple range test (DMRT)

Table.2 Efficacy of *Pseudomonas fluorescens* against *Fusarium oxysporum* f. sp. *lycopersici* (Fol₃) (Poison food technique)

S. No	Isolates	Mycelial growth(mm)							
		10%	Percent inhibition over control	20%	Percent inhibition over control	30%	Percent inhibition over control	40%	Percent inhibition over control
1.	Pf ₁	54.84 ^e	39.06	31.43 ^e	65.07	21.43 ^e	76.18	11.79 ^e	86.90
2.	Pf ₂	59.76 ^h	33.60	35.46 ^h	60.60	25.92 ^h	71.20	14.89 ^h	83.45
3.	Pf ₃	60.56 ⁱ	32.71	37.61 ⁱ	58.21	26.46 ⁱ	70.60	15.36 ⁱ	82.93
4.	Pf ₄	61.26 ^j	31.93	38.31 ^j	57.43	27.62 ^j	69.31	16.46 ^j	81.71
5.	Pf ₅	45.89 ^a	49.01	28.45 ^a	68.38	16.78 ^a	81.35	8.46 ^a	90.60
6.	Pf ₆	47.46 ^b	47.26	28.79 ^b	68.01	17.89 ^b	80.12	9.23 ^b	89.74
7.	Pf ₇	55.78 ^f	38.02	32.78 ^f	63.57	22.71 ^f	74.76	12.63 ^f	85.96.
8.	Pf ₈	53.12 ^d	40.97	30.65 ^d	65.94	21.35 ^d	76.27	10.96 ^d	87.82
9.	Pf ₉	51.23 ^c	43.07	29.78 ^c	66.91	19.67 ^c	78.14	10.25 ^c	88.61
10.	Pf ₁₀	57.56 ^g	36.04	34.12 ^g	62.08	23.41 ^g	73.98	13.64 ^g	84.84
11.	Control	90.00	-	90.00	-	90.00	-	90.00	-

* Mean of three replications; * In a column, means followed by a common letter are not significantly differ at 5% level by Duncan's multiple range test (DMRT)

Table.3 In vitro efficacy of *Bacillus subtilis* against *Fusarium oxysporum* f. sp. *Lycopersici* (Fol₃) (Dual culture)

S. No	Isolates	Locality	Mycelial growth (mm)	Percent inhibition over control
1.	Bs1	Hamumantheertham	51.78 ^c	42.46
2.	Bs2	Irumathur	62.16 ^g	30.93
3.	Bs3	Uthangarai	72.65 ^j	19.27
4.	Bs4	Thippampatti	68.23 ⁱ	24.18
5.	Bs5	Puthoor	43.48 ^b	51.68
6.	Bs6	Arasur	32.63 ^a	63.74
7.	Bs7	Kollanaikanoor	54.36 ^d	39.60
8.	Bs8	Mittapalli	59.45 ^e	33.94
9.	Bs9	Kodamandapatti	60.12 ^f	33.20
10.	Bs10	Mathur	65.76 ^h	29.93
11.	Control		90.00	-

* Mean of three replications

* In a column, means followed by a common letter are not significantly differ at 5% level by Duncan's multiple range test (DMRT)

Table.4 Efficacy of *Bacillus subtilis* against *Fusarium oxysporum* f. sp. *lycopersici* (Fol₃) (Poison food technique)

S. No	Isolates	Mycelial growth(mm)							
		10%	Percent inhibition over control	20%	Percent inhibition over control	30%	Percent inhibition over control	40%	Percent inhibition over control
1.	Bs1	43.12 ^c	52.08	34.48 ^c	61.68	18.45 ^c	79.50	7.23 ^c	91.96
2.	Bs2	52.64 ^g	41.51	41.78 ^g	53.57	26.87 ^g	70.14	13.47 ^g	85.03
3.	Bs3	56.35 ^j	37.38	46.12 ^j	48.75	30.61 ^j	65.98.	18.46 ^j	79.48
4.	Bs4	55.73 ⁱ	38.07	45.36 ⁱ	49.60	28.78 ⁱ	68.02	17.74 ⁱ	80.28
5.	Bs5	42.65 ^b	52.61	32.76 ^b	63.60	17.65 ^b	80.38	5.45 ^b	93.94
6.	Bs6	40.48 ^a	55.02	31.23 ^a	65.30	15.78 ^a	82.46	3.2 ³	96.41
7.	Bs7	45.35 ^d	49.61	35.62 ^d	60.42	22.34 ^d	75.17	9.46 ^d	89.48
8.	Bs8	46.89 ^e	47.90	37.13 ^e	58.74	23.16 ^e	74.26	11.34 ^e	87.40
9.	Bs9	49.56 ^f	44.93	39.34 ^f	56.28	24.64 ^f	72.62	12.16 ^f	86.48
10.	Bs10	53.76 ^h	40.26	45.34 ^h	49.62	27.47 ^h	69.47	13.34 ^h	85.17
11.	control	90.00		-	-	-	-	-	-

* Mean of three replications

* In a column, means followed by a common letter are not significantly differ at 5% level by Duncan's multiple range test (DMRT)

In vitro efficacy of *Bacillus subtilis* against *Fusarium oxysporum* f. sp. *lycopersici*

Among the isolates, the isolate Bs₆ collected from Arasur showed the maximum inhibition and significantly inhibited the growth of *F. oxysporum* f. sp. *lycopersici* (32.63 mm), which was 63.74 per cent reduction on the growth of the pathogen when compared to control. In poison food technique the maximum reduction in the growth of mycelium is noticed in the isolate Bs₆ with 40.48mm, 31.23mm, 15.78mm and 3.23mm with percent inhibition of 55.02 %, 65.30%, 82.46% and 96.41% at 10%, 20%, 30% and 40% respectively.

The mode of antagonism generally observed with *Bacillus* spp. is antibiosis (Edwards *et al.*, 1994). This is supported by reports that most *Bacillus* spp. produce many antibiotics such as bacillomycin, fengycin, mycosubtilin and zwittermicin, which are all effective at suppressing growth of target pathogens *in vitro* (Pal and Gardener, 2006). This evidence allows the assumption that antibiotics are related to the inhibition of the test pathogen observed in this study. Volatiles from *B. megaterium* KU143 (5-methyl-2-phenyl-1H-indole from *B. megaterium* KU143 and 2-butyl 1-octanal, dimethyl disulfide, 2-isopropyl-5-methyl-1-heptanol) and *Trichoderma* (2-butyl 1-octanal) inhibited mycelial growth, sporulation, conidial germination, and aflatoxin production by *A. flavus* on media and rice grains (Mannaa *et al.*, 2017). Thus the present findings corroborates with earlier works.

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How to cite this article:

Murugavel, K. and Kannan, R. 2020. In vitro efficacy of *Pseudomonas fluorescens* and *Bacillus subtilis* against *Fusarium oxysporum* f. sp. *lycopersici*. *Int.J.Curr.Microbiol.App.Sci*. 9(07): 1569-1576. doi: <https://doi.org/10.20546/ijemas.2020.907.182>