SHORT COMMUNICATION

Evaluation of *Pseudomonas fluorescens* isolates for the management of *Fusarium oxysporum* f.sp. *lycopersici* and *Rhizoctonia solani* causing wilt complex in tomato

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ABSTRACT: Twenty-five isolates of *Pseudomonas fluorescens* were isolated from healthy plants of tomato on Kings'B medium, for exploring their bio-efficacy potential against *Fusarium oxysporum* f.sp. *lycopersici* and *Rhizoctonia solani*, responsible for wilt complex disease. Out of all, six isolates (I-7, I-15, I-18, I-23, I-24 and I-25) significantly inhibited the growth of both fungal pathogens under laboratory conditions. Under green house conditions, bacterization of tomato seeds with these six isolates of *P. fluorescens* in pathogen infested soil significantly reduced the incidence of disease caused by *F. oxysporum* f.sp. *lycopersici* and *R. solani*, with maximum (12.50 and 10.41%) by isolate I-23, and simultaneously increased the plant growth parameters. Application of isolate I-23 also resulted in maximum per cent germination, shoot length and root length of 80.00 and 77.50 per cent and 54 and 38 cm and 16.27 and 11.97 cm, respectively as compared to their untreated control.

Keywords: Bio-efficacy, *Fusarium oxysporum* f.sp. *lycopersici*, *Pseudomonas fluorescens*, *Rhizoctonia solani*, tomato

Among the different biotic factors, wilt complex disease caused by *Fusarium oxysporum* f.sp. *lycopersici* and *Rhizoctonia solani* is responsible for plant mortality and significant reduction in fruit yield of tomato (*Solanum lycopersicum* L.), which is the third most cultivated vegetable in the India. Management of soil-borne diseases has always been problematic, though soil solarization, crop rotation and mixed cropping have been suggested as the ways to eliminate soil borne pathogens. Application of synthetic fungicides considerably reduce wilt incidence, however, their use is expensive and environmentally undesirable. Management of plant diseases using antagonistic rhizobacteria is an effective and eco-friendly alternative to the use of synthetic chemicals. *Pseudomonas fluorescens* has been extensively used against different soil borne pathogens in several crops (Gopi *et al.*, 2016). Mechanisms involved in disease suppression by fluorescent pseudomonads include competition for nutrients and space, antibiosis, production of siderophores, induction of systemic resistance, plant growth promotion, production of lytic enzymes and detoxifying enzymes (Nagarajkumar *et al.*, 2005). The present study was undertaken with an aim to explore the possibilities of indigenous *P. fluorescens* isolates for their bio-efficacy and plant growth promotion activities in tomato crop.

Tomato plants exhibited symptoms of disintegration of stem, collar and wilting were collected from different

locations of Jammu province to isolate *Fusarium oxysporum* f.sp. *lycopersici* and *Rhizoctonia solani*. Bits of approximately 2 to 3 mm in size were cut, surface sterilized, transferred aseptically onto petriplates having pre-poured PDA + streptomycin sulphate and then incubated at 25±2°C in BOD incubator. The fungal growth obtained from diseased plant parts was purified using hyphal tip method. Morphological examinations and cultural characteristics of the isolated fungi were recorded and the observations compared with the standard text (Booth, 1971; Sneh *et al.*, 1991). Frequency of the pathogens associated with the diseased samples of tomato crop was calculated by assessing the recovery of each pathogen from the total number of isolations made.

Twenty-five isolates of *P. fluorescens* based on morphological and biochemical tests (Schaad *et al.*, 2001) were isolated from the rhizosphere of tomato fields of the University Research Farm, by serial dilution method and were tested against the isolated fungal pathogens (*F. oxysporum* f.sp. *lycopersici* and *R. solani*) by using dual culture technique (Dhingra and Sinclair, 1995). Under pot experiment, activities of six selected *P. fluorescens* isolates were conducted to assess the growth promoting potential and disease suppression. The soil was mixed with the FYM in 2:1 ratio and sterilized with 5 per cent formalin by thoroughly mixing and then covering it with a polyethylene sheet for one week. The pathogens were grown in potato dextrose broth for 15

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Table 1. Frequency of pathogens isolated from the infected tomato plants

Location	Per cent frequency of the pathogens recovered		
	<i>Fusarium oxysporum</i> f.sp. <i>lycopersici</i>	<i>Rhizoctonia solani</i>	<i>F. oxysporum</i> f.sp. <i>lycopersici</i> + <i>R. solani</i>
Poni Chak	56.00	32.00	12.00
Akalpur	48.00	30.00	22.00
Pinjore	40.00	16.00	29.00
Marh	61.00	25.00	14.00
Arnia	42.00	31.00	27.00
Dab Sudan	55.00	20.00	25.00
Mean	50.33	25.67	21.50
Range	40.00- 61.00	16.00- 32.00	12.00-29.00
SE _m ±	3.27	2.46	2.61
CD(P=0.05)	10.18	7.66	8.14

Table 2. *In vitro* evaluation of *Pseudomonas fluorescens* isolates against *F. oxysporum* f.sp. *lycopersici* and *R. solani* by dual culture technique

Isolate	Mean radial growth (mm)		Inhibition over control (%)	
	<i>F. oxysporum</i> f.sp. <i>lycopersici</i>	<i>R. solani</i>	<i>F. oxysporum</i> f.sp. <i>lycopersici</i>	<i>R. solani</i>
I-1	40.00	38.00	55.55	57.77
I-2	65.33	65.66	27.41	27.04
I-3	44.33	48.00	50.74	46.66
I-4	71.33	70.33	21.11	21.85
I-5	60.33	65.66	32.97	27.04
I-6	69.66	67.33	22.60	25.18
I-7	34.33	31.33	61.85	65.18
I-8	69.00	67.66	23.33	24.82
I-9	48.66	50.33	45.93	44.07
I-10	58.00	66.00	35.56	26.66
I-11	46.66	48.00	48.15	46.66
I-12	64.66	72.00	28.15	20.00
I-13	68.66	67.00	23.71	25.55
I-14	63.00	55.00	30.00	29.26
I-15	32.00	31.00	64.44	65.55
I-16	45.33	48.33	32.22	46.30
I-17	66.66	65.33	25.93	27.41
I-18	32.66	37.33	63.71	58.52
I-19	39.66	40.33	55.93	55.18
I-20	74.66	72.00	17.04	20.00
I-21	63.66	57.00	29.26	36.66
I-22	46.00	39.66	48.88	55.93
I-23	30.00	30.66	66.66	65.93
I-24	44.00	35.00	51.11	61.11
I-25	37.66	31.00	58.15	65.55
Control	90.00	90.00	0.00	0.00
SE _m ±	1.90	2.15		
CD(P= 0.05)	5.40	6.13		

days at 28±2°C in BOD. The cfu/ml was maintained @ 1.2×10^7 for *F. oxysporum* f.sp. *lycopersici* and 8×10^5 for *R. solani* with the help of hemocytometer. The pots filled with sterilized soils were inoculated with the pathogens prior to sowing of tomato seed (before one week). Tomato seeds (Heem Sohna) were surface sterilized, dipped in suspension of isolates of *P. fluorescens* viz., I-07, I-15, I-18, I-23, I-24 and I-25, grown on nutrient agar medium for 48 hours, harvested with sterile distilled water and

adjusted to the concentration of 1×10^8 cfu/ml. Five seeds were planted in each pot and subsequently inoculated with each isolate. Each treatment was replicated five times. Observations regarding disease incidence, germination (%) and shoot and root length (cm) were recorded for each isolate.

Fusarium oxysporum f.sp. *lycopersici* and *R. solani* were the predominant pathogens isolated from the

Table 3. Effect of seed treatment of *P. fluorescens* isolates on disease incidence and growth of tomato plants

Treatment	Disease incidence (%)	Germination (%)	Shoot length (cm)	Root length (cm)
F+I-7	14.58	67.50	32.33	7.13
F+I-15	16.67	72.33	33.67	10.90
F+I-18	27.08	60.50	29.67	10.50
F+I-23	12.50	80.00	54.00	16.27
F+I-24	25.00	65.00	35.00	15.80
F+I-25	20.83	75.00	47.33	11.53
F	72.91	37.50	22.67	8.88
R+I-7	12.16	60.00	26.67	7.70
R+I-15	27.08	72.33	33.67	9.87
R+I-18	16.67	75.00	25.67	8.67
R+I-23	10.41	77.50	38.00	11.97
R+I-24	18.75	70.50	37.33	7.17
R+I-25	27.08	75.00	35.33	7.33
R	62.50	45.00	19.67	3.74
SE _m ±	2.21	4.74	4.97	1.43
CD(0.05)	6.41	13.77	14.42	4.14

F: *F. oxysporum* f.sp. *lycopersici*; R: *R. solani*; I: Isolate of *P. fluorescens*

infected tomato plants collected from different locations of Jammu province. The two pathogens were found either alone or in combination with each other. The data presented in Table 1 indicated that *F. oxysporum* f.sp. *lycopersici* was the most dominant pathogen with an average occurrence of 50.33 per cent, followed by *R. solani* (25.67%), whereas, the isolation of both the pathogens in the same infected sample were observed in 21.50 per cent. The data indicated that frequency of the occurrence of the pathogens varied significantly from one location to the other. The recovery of the *F. oxysporum* f.sp. *lycopersici* was maximum from the disease samples collected from Marh (61.00%), PoniChak (56.00%) and Dab Sudan (55.00%), whereas, that of *R. solani* it was 32.00, 31.00, 30.00, and 25.00 per cent from Poni Chak, Arnia, Akalpur and Marh, respectively. The combined occurrence of the two pathogens was observed maximum from Pinjore (29.00%) followed by Arnia (27.00%) and Dab Sudan (25.00%). Bharat and Sharma (2014) also recorded highest incidence of the wilt at Kadriyana (27.65%) in district Hamirpur followed by Nauni (25.56%) in district Solan, whereas lowest incidence was recorded at Dharot (9.90%) in district Hamirpur followed by Gassour (12.50%) in district Bilaspur of Himachal Pradesh. Prevalence of higher temperature (35-40°C), continuous cropping of tomato round the year were the main conditions responsible for the multiplication and spread of soil borne diseases especially Fusarium wilt (Charoenporn *et al.*, 2010).

Under dual culture technique, isolate I-23 exhibited minimum radial growth and maximum growth inhibition of *F. oxysporum* f.sp. *lycopersici* and *R. solani* (30.00 and 30.66 mm and 66.66 and 65.93 per cent, respectively), over the control. Isolate I-23 was followed by isolate I-15 with 32.00 mm (*F. oxysporum* f.sp. *lycopersici*) and 31.00 mm (*R. solani*) radial growth having 64.44 and 65.55 per

cent inhibition, respectively (Table 2). Inhibition of mycelial growth of *F. oxysporum* f.sp. *lycopersici* and *R. solani* could be due to nutritional competition and/or colonization of fungal hyphae by the bacteria (Asha *et al.*, 2011). *P. fluorescens* is also known to secrete antifungal metabolites such as lipopeptide cyclic, amphisin and several other hydrolytic enzymes such as endochitinase, chitinase, β -1, 4 glucanase, which have an inhibitory effect on cell wall of fungal pathogens (Saad, 2006).

Out of total, the selected *P. fluorescens* isolates (I-7, I-15, I-18, I-23, I-24 and I-25) significantly reduced the disease incidence caused by *F. oxysporum* f.sp. *lycopersici* and *R. solani*, over untreated control (Table 3). Application of isolate I-23 resulted in minimum disease incidence of 12.50 and 10.41 per cent against *F. oxysporum* f.sp. *lycopersici* and *R. solani*, respectively. It was followed by isolate I-7 having 14.58 and 12.16 and 16.67 per cent disease incidence by *F. oxysporum* f.sp. *lycopersici* and *R. solani*, respectively. Maximum seed germination of 80.00 per cent was recorded in F+I-23 treatment followed by R+I-23 (77.50%). Maximum shoot and root length (54.00 and 16.27cm) was recorded in F+I-23 followed by F+I-25 and F+I-24. Similar results were observed where seed bacterization with bacterial antagonists improved seed germination and resulted in significantly higher vigour index (Ardebili *et al.*, 2011; Sneh *et al.*, 1991). Moreover, better root system (increased root length) in seeds treated with *P. fluorescens* might have tolerated or escaped root infections, facilitating an active absorption of nutrients there by promoting the plant growth and health.

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