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Fusarium Wilt of Tomato Managed by *Pseudomonas fluorescens*

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A B S T R A C T

The present studies were undertaken to investigate the effect of native isolates of *Pseudomonas fluorescens* (Pf) against Fusarium wilt of tomato. All Pf isolates showed similar results in gram staining (Gram negative), motility, starch hydrolysis, gelatin liquefaction, fluorescent pigment (all are positive). All the *P. fluorescens* isolates also produced positive results in production of IAA. Among the various isolates, the highest inhibition was shown by isolate Pf_5 and it was followed by Pf_3. The least inhibition was recorded by isolate Pf_2. Also, the effective isolate Pf_5 recorded the maximum inhibition of mycelial dry weight under liquid and solid medium. The comparison fungicide Carbendazim 50% WP @ 0.1% conc. showed the highest percent inhibition over control.

***Key words*:** Tomato, Fusarium wilt, Antagonistic agent, *Pseudomonas fluorescens*

Tomato (*Solanum lycopersicum* L.) is one of the widely cultivated commercial vegetable crop throughout the world [1] . Its popularity is due to the excellent source of vitamins, antioxidants, micronutrients, phosphorus, iron and its curative properties [2]. The world production of tomato is 170.80 million tonnes and China ranks first in tomato production worldwide with the contribution of 31% of global production followed by India. The *Fusarium* wilt caused by *Fusarium oxysporum* f.sp. *lycopersici* is an economically important and most destructive disease of tomato crop [3]. The bacterial antagonist *Pseudomonas fluorescens* also effectively manages the *Fusarium* wilt [4] .

MATERIALS AND METHODS

Isolation of native antagonists from tomato rhizosphere soil Pseudomonas fluorescens

Fluorescent bacteria were isolated from the rhizosphere soil of tomato plants following serial dilution techniques suggested by [5]. 1 g of soil was serial diluted up to 10^{-6}. One ml of sample taken from the dilutions of 10^{-5} and 10^{-6} respectively was poured into the king's B medium and incubated at room temperature for 48 hours. Representative colonies were detected under UV light. These colonies were purified by single colony method. The five isolates were designated as Pf1 - Pf5.

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Biochemical test for Pseudomonas spp

For the identification of *P. fluorescens*, certain biochemical test was conducted according to Bergey´s manual for determinative bacteriology [6].

Gram staining [7].
Starch hydrolysis [8].
Gelatine liquefaction [8].
Fluorescent pigmentation [5].
Estimation of IAA [9]
Extraction of siderophore from the medium [10].
Hydrogen Cyanide (HCN) production [11]

Efficacy of antagonists
Effect on P. fluorescens isolates against F. oxysporum f.sp. lycopersici (Dual culture)

The antagonistic effect of *P. fluorescens* isolates (Pf1 - Pf5) against *F. oxysporum* f.sp. *lycopersici* was tested through dual culture techniques [12]. In this method 9mm mycelia disc from seven days old culture of F. *oxysporum* f.sp. l*ycopersici* was inoculated at one edge of Petri dish containing 15 ml solidified PDA medium. On the second day, the two days old culture of *P. fluorescens* was streaked on the opposite side of the plate. Pathogen alone inoculated at one edge of the Petri dish served as control. After three days of incubation the radial growth of pathogen and inhibition zone were recorded and percent inhibition over control was estimated by using the formula [13].

$$\text{Percent inhibition (I)} = C - T / C \times 100$$

Where;
C - Mycelia growth of pathogen in control (mm)
T - Mycelia growth of pathogen in dual plate (mm)

Effect of cultural filtrate of P. fluorescens on the mycelia growth and mycelia dry weight of Fusarium oxysporum f.sp. lycopersici

Pseudomonas fluorescens culture was inoculated into 50 ml of sterilized King's B broth and kept in a rotary shaker at 100 rpm for 48 hours. The culture broth was filtered through bacteriological filter. The supernatant solution was sterilized and added to the sterilized PDA medium concentration @ 10, 20, 30, 40, 50 percent conc. The PDA medium without culture filtrate served as control. The media were poured in to Petri dishes separately @ 15 ml and allowed to solidify. Seven days old culture of *Fusarium oxysporum* f.sp. *lycopersici* (7mm) was placed in the center of each plate. Carbendazim @ 0.1% of carbendazim was used for comparison. The colony growth of *Fusarium oxysporum* f.sp. *lycopersici* was recorded when colony growth fully covered the control plates.

RESULTS AND DISCUSSION

Biochemical test for Pseudomonas fluorescens native isolates

The five selected native *P. fluorescens* isolates were designated as Pf_1 - Pf_5. The most effective isolate of *P. fluorescens was* identified by a series of biochemical test and the data are presented in (Table 1). All Pf isolates showed similar results in Gram staining (Gram negative), Motality (Positive), starch hydrolysis (positive), Gelatin liquifaction (positive), Fluorescent pigment (positive). All the *P. fluorescens* isolates produced positive results in production of IAA.

Table 1 Biochemical test for *Pseudomonas fluorescens* native isolates

Parameter	Isolates of *P. fluorescens*				
	Pf_1	Pf_2	Pf_3	Pf_4	Pf_5
Gram staining	Negative	Negative	Negative	Negative	Negative
Motility	Positive	Positive	Positive	Positive	Positive
Starch hydrolysis	Negative	Negative	Negative	Negative	Negative
Gelatin liquefaction	Positive	Positive	Positive	Positive	Positive
Fluorescent pigment	Positive	Positive	Positive	Positive	Positive
Estimation of IAA(µg/ml)	2.4	3.5	2.7	3.1	3.6
Siderophore production (Hydroxamate)	0.81	0.86	0.82	0.84	0.89
Hydrogen cyanide production	7.48	7.95	7.63	8.06	8.15

Table 2 Antagonistic activity of native *Pseudomonas fluorescens* isolates against *F. oxysporum* f.sp. *lycopersici* (Dual culture Techniques)

Treatment No.	Isolate	Mycelia growth of *F. oxysporum* f.sp. *lycopersici* (mm)	Inhibition zone (mm)	Per cent inhibition over control (%)
T_1	Pf_1	67.20^{d}	5.45	25.33
T_2	Pf_2	72.82^{e}	4.21	19.08
T_3	Pf_3	52.57^{b}	7.02	41.58
T_4	Pf_4	60.43^{c}	6.50	32.85
T_5	Pf_5	41.80^{a}	7.56	53.55
T_6	Control	90.00^{f}	---	---

*****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)

Antagonistic activity of native Pseudomonas fluorescens isolates against F. oxysporum f.sp. lycopersici by dual culture techniques

The antagonistic activity of 5 native isolates of *P. fluorescens* against Fol was assessed through dual culture technique and the data recorded in (Table 2). The highest inhibition was shown by isolate Pf_5 which inhibited the mycelia growth of Fol (41.80 mm) and recorded a percent inhibition over control (53.55%) followed by Pf_3 (52.57 mm and 41.58%), Pf_4 (60.43 mm and 32.85%). The least inhibition was recorded by isolate Pf_2 (72.82 mm and 19.08%). The isolate Pf_5 proved its supremacy by recording an inhibition zone of 7.56. Hence, the isolate Pf5 was used for further experiments.

Table 3 Effect of cultural filtrate of *P. fluorescens* on the mycelia growth and mycelia dry weight of *F. oxysporum* f. sp. *lycopersici*

Treatment No.	Concentration of culture filtrate %	Solid medium		Liquid medium	
		Mycelia growth (mm)	Per cent inhibition over control	Mycelia dry weight(mg)	Per cent inhibition over control
T_1	10	50.46^{f}	43.90	176.40^{f}	34.81
T_2	20	41.43^{e}	53.96	158.23^{e}	41.53
T_3	30	33.78^{d}	62.46	86.72^{d}	67.95
T_4	40	28.96^{c}	67.82	51.68^{c}	80.90
T_5	50	18.72^{b}	79.20	20.21^{b}	92.53
T_6	Carbendazim 50%WP @0.1	0.00^{a}	100.00	4.07^{a}	98.49
T_7	Control	90.00^{g}	---	270.62^{g}	---

Effect of cultural filtrate of Pseudomonas fluorescens on the mycelia growth and mycelia dry weight of Fusarium oxysporum f. sp. lycopersici

The data presented in the (Table 3) revealed the efficacy of Pf_5 isolate against test pathogen Fol by Poisoned food technique. In solid medium, cultural filtrates at 10, 20, 30, 40, 50% concentrations recorded the mycelia growth of the fungus viz. (50.46, 41.43, 33.78, 28.96, 18.72 mm) and percent inhibition over control (43.90, 53.96, 62.46, 67.82, 79.20%) respectively. In liquid medium, the maximum inhibition of biomass production was found in 50% concentration where the dry weight of the mycelium was 20.21 mg when compared to control 270.62mg and the percent inhibition over control was found to be 92.53%. The chemical control Carbendazim 50% WP @ 0.1% conc. showed the highest percent inhibition over control (98.49%). *Pseudomonas* spp effectively inhibited the *Fusarium oxysporum* f.sp *ciceri* by dual culture technique [14]. *P. putida* (P10) and *Pseudomonas aueroginosa* (P12) showed the highest inhibition percentage (40.30% and 44.9%) respectively. *T. viride* isolate TV3 showed maximum inhibition percentage (48.0%) against Fol followed by other isolates TH 23, TH 19, TH 32, TH 13, TH 7 having inhibition percentage ranging from (30.0% to 45%). Harshita (2018) also reported that Fol was effectively controlled by *Pseudomonas fluorescens* forming an inhibition zone of 25.6mm [4].

CONCLUSION

From the aforementioned investigation it could be conclude that the highest inhibition was shown by isolate Pf_5 and it was followed by Pf_3. The least inhibition was recorded by isolate Pf_2. Also, the effective isolate Pf_5 recorded the maximum inhibition of mycelial dry weight under liquid and solid medium. The comparison fungicide carbendazim 50% WP @ 0.1% conc. showed the highest percent inhibition over control.

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