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National Academy of Agricultural Science (NAAS) Rating : 3. 03

In Vitro Evaluation of *Pseudomonas Fluorescens* Strains Against *Fusarium Oxysporum* f.sp. *Lycopersici* Causing Wilt Disease of Tomato

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ABSTRACT: *Tomato is one of the important vegetable crops cultivated extensively worldwide. Among the tomato diseses, soil borne disease namely Fusarium wilt is playing a major role in drastic yield reduction. Pseudomonas fluorescens is a potential biocontrol agent for management of soil borne diseases of crop plants. In the present work, different strains of Pseudomonas fluorescens were isolated from different tomato growing areas of Tamil Nadu. An experiments was conducted to identiy effective strains of Pseudomonas against Fusarium oxysporum f.sp. lycopersici. The result of the experiment revealed that, P. fluorescens strain Pf12 isolated from Udumalipet village of Tirupur district showed higher inhibition of mycelial growth of the pathogen. This was followed by Pf 3, Pf 9, Pf 13 and Pf 14. Hence, the current study suggests that, P. fluorescens (Pf12) can also be used as biocontrol agent to manage the Fusarium wilt disease.*

Keywords: *Biocontrol agent, Tomato Fusarium wilt, In vitro screening, Pseudomonas.*

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crop and cultivated for its fleshy fruits, high nutrient value, rich source of mineral, vitamins, essential amino acides, dietary fibres and high yield. This tomato crop is affected by various fungal, bacterial and viral diseases (Doolittle 1948). Among those diseases the *Fusarium* wilt disease caused by *Fusarium oxysporum* f.sp. *lycopersici* considerable losses on tomato plants. Management of diseases in tomato is widely practiced using fungicides (Singh *et al.,* 2001). However, continuous usage of such fungicides led to the development of resistance by the pathogen and causes environmental pollution as well as creates hazards to human healths (Rai *et al.,* 2000). Under these circumstances, inclusion of management strategies using plant growth promoting rhizobacteria (PGPR) namely *Pseudomonas fluorescens* played major role in managing the pathogen in crop plants are well reported (Harish *et al.*, 2008). They survive in seed or soil, multiply in the spermosphere in response to seed exudates rich in carbohydrates and amino acids (Kloepper *et al.*, 1992) attach to root surface (Suslow, 1980) and become endophytic by colonizing in root cortex region. The use of Fluorescent pseudomonads for controlling soil borne plant diseases has been well documented (Radjacommare *et al.,* 2002; Ramamoorthy *et al.,* 2002). PGPR exhibits multiple numbers of mechanisms to promote plant growth and to serve as potential biocontrol agent. Generally, it has various activities *viz.*,

(I) fixing atmospheric nitrogen and supply it to plants

(II) synthesizing various phytohormones including auxins and cytokinins

(III) providing mechanisms for the solubilization of minerals such as phosphorus

(IV) antibiotic synthesis (Haas and Defago, 2005)

(V) secretion of iron binding siderophores to obtain soluble iron from the soil and provide it to a plant and thereby deprive fungal pathogens in the vicinity of soluble iron (Dowling *et al.*, 1994)

(VI) production of low molecular weight metabolites such as hydrogen cyanide with antifungal activity (Dowling and O'Gara, 1994)

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(VII) production of defense enzymes including chitinase, â-1,3-glucanase, protease and lipase which cause lysis of some fungal cells

(VIII) production of oxidative stress enzymes such as catalases, superoxide dismutases and peroxidases for scavenging active oxygen species

(IX) competition for nutrients and niches on the root surface and

(X) lowering the production of stress ethylene in plants with the enzyme ACC deaminase (Saravanakumar and Samiyappan, 2007).

P. fluorescens inhibiting or displacing soil borne pathogens at the root-soil interface, thereby protecting the root health has been studied by various workers in annual crop plants such as cotton, potato, tobacaco, flax, cucumber, sunflower, wheat and rice (Weller *et al.*, 2002). Protective effect in chilli was observed due to the application of rhizobacterial strain *P. ûuorescens* (Pf1) against *Fusarium* disease (Sundaramoorthy *et al.*, 2012). Antagonistic effects of six isolates of *Pseudomonas* were identified in chickpea against *F. oxysporum* f. sp. *ciceris in vitro* and *in vivo* (Karimi *et al.*, 2012). Liquid formulation of *P. fluorescens* Pf1 exhibited higher induction of defense enzymes and reduced the incidence of tomato *Fusarium* wilt disease (Manikandan and Raguchander, 2014). Enhancement of resistance and retardation of *C. musae* was observed in banana plants treated with water in oil based PGPR formulation of *P. ûuorescens* (FP7) (Mohammed Faisal *et al.*, 2014a). Application of *P. fluorescens* liquid formulation through drip system significantly reduced the *Fusarium* wilt complex in banana under field conditions (Selvaraj *et al.*, 2014).

With this background information, the present investigation was undertaken to evaluate the different strains of Pseudomonas fluorescens against *Fusarium* wilt of tomato under *in vitro* conditions.

MATERIALS AND METHODS

Isolation of Different Strains of *Pseudomonas fluorescens* from Rhizosphere Soils of Tomato

Rhizosphere colonizing *Pseudomonas* spp. were isolated from different rhizosphere soils of tomato growing of regions of Tamil Nadu. The soil particles tightly adhered with tomato root surface were removed and suspended in 10ml sterile distilled water. After serially diluted (upto 10^6) one ml of suspension from each 10^3 to 10^6 dilutions was transferred to sterile Petri dishes containing King's B(KB) for the isolation of *Pseudomonas* spp respectively. Observations were taken after one day for the presence of bacterial antagonists. The bacterial antagonists were further purified on their respective media and compared with the isolates maintained in laboratory.

Isolation of Pathogen and Pathogenicity Test of *Fusarium Oxysporum* f.sp. *Lycopersici*

The soil borne pathogen, *Fusarium oxysporum* f. sp. *lycopersici* was isolated by tissue segment method on potato dextrose medium. Infected stems and roots were cut into small pieces of 1 to 1.5cm, surface sterilized with 1 per cent sodium hypocholorite for one min. and washed in sterile distilled water thrice and then placed in Petri plate containing sterilized solidified potato dextrose agar (PDA) medium. The hyphal tips of fungi growing from the pieces were transferred aseptically to PDA slants for further studies (Rangaswami, 2005).

The fungi isolated were multiplied on sand maize medium (Riker and Riker, 1936). Sand and ground maize seeds were mixed in the ration of 19:1, moistened to 50 percent moisture content, filled in 500 ml conical flask and autoclaved at 1.4 kg cm^2 for two hours. The 8mm disc of pure culture of the fungus was incubated at room temperature (28 ± 2°C) for 14 days and used for the pathogenicity. The fungus multiplied on sand maize medium was incorporated into the sterilized soil at the rate of 5 percent (w/w). Three month's old tomato plants were inoculated with the pathogen and observed for symptoms expression up to 7 days after inoculation (DAI) under glasshouse condition.

In Vitro Testing of *Pseudomonas Fluorescens* on Inhibition of Mycelial Growth of *Fusarium Oxysporum* f.sp. *Lycopersici*

A nine mm mycelial disc of the tomato wilt pathogen *Fusarium oxysoporum* f.sp. *lycopersici* was placed in the centre of the Petriplate. Sterile Whatman No. 40 filter paper discs with six mm dia were placed 1cm away from the edge at four sides centering around the fungal disc. Twenty five micro litres of broth cultures of different *P. fluorescens* culture were dropped over the filter paper discs. The plates were incubated at room temperature (28 ± 2°C) and the observations were taken after five days for the presence of inhibition zone over the pathogen and nearer to the

Table 1
Isolation of *Pseudomonas fluorescens* from different places of tomato growing areas of Tamil Nadu

S. No.	*Strains*	*Isolate*	*Place*	*District*
1.	*Pseudomonas fluorescens*	Pf1	Kinathukadavu	Coimbatore
2.	*Pseudomonas fluorescens*	Pf2	Thazhiyur	Coimbatore
3.	*Pseudomonas fluorescens*	Pf3	Thondamuthur	Coimbatore
4.	*Pseudomonas fluorescens*	Pf4	Karamadai	Coimbatore
5.	*Pseudomonas fluorescens*	Pf5	Malumichaipatti	Coimbatore
6.	*Pseudomonas fluorescens*	Pf6	Pollachi	Coimbatore
7.	*Pseudomonas fluorescens*	Pf7	Kattukotai	Villupuram
8.	*Pseudomonas fluorescens*	Pf8	Veerachozhapuram	Villupuram
9.	*Pseudomonas fluorescens*	Pf9	Kadhuaruthanmedu	Villupuram
10.	*Pseudomonas fluorescens*	Pf10	Chinnaselam	Villupuram
11.	*Pseudomonas fluorescens*	Pf11	Kallakurichi	Villupuram
12.	*Pseudomonas fluorescens*	Pf12	Udumalaipet	Tirupur
13.	*Pseudomonas fluorescens*	Pf13	C.K.Valasu	Dindigul
14.	*Pseudomonas fluorescens*	Pf14	Dindigal	Dindigul
15.	*Pseudomonas fluorescens*	Pf15	Pudukkottai	Poudukotti

bacterial spot. Control was maintained with the sterile distilled water instead of bacterial inoculum.

RESULTS

Isolation of Endophytic and Plant Growth Promoting Rhizobacteria

Totally fifteen strains of *Psedudomonas spp* were isolated from rhizosphere of soils of tomato from different districts of Tamil Nadu *viz*., Coimbatore, Villupuram, Tirupur, Dindigul and Pudukottai (Table 1). All the strains were identified as *Pseudomonas fluorescens* based on the morphological and biochemical characters. The strains were designated as Pf 1 to Pf 15.

Cultural Characters of the Pathogen

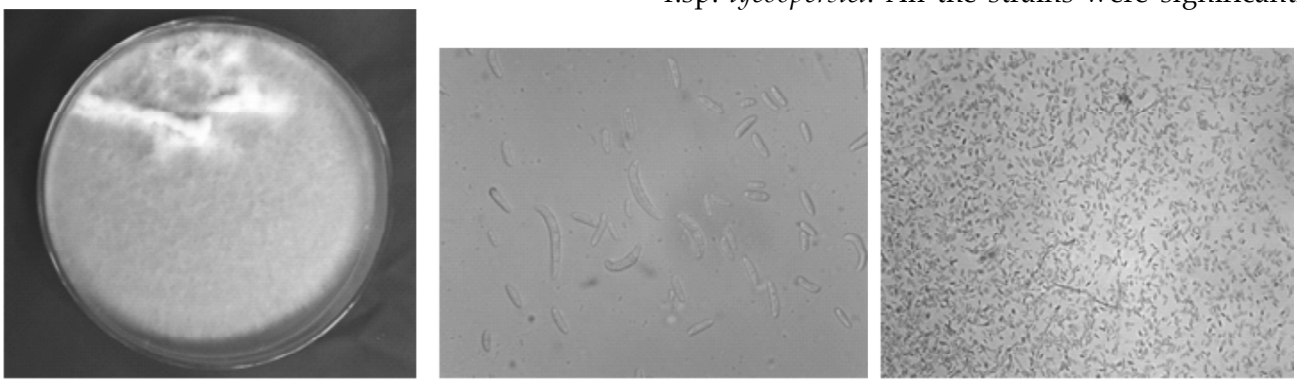
The pathogen was isolated from the infected tomato roots using potato dextrose agar medium (PDA). In the laboratory, *Fusarium* isolate produced a dense mycelial growth on PDA. The pathogen *F. oxysporum* f.sp *lycopersici* was identified microscopically. Macroconidia was sickle shaped, hyaline and multicelled with 3 septation. Micro conidia were small, oval shaped, single or bicelled. The number of micro conidia was more as compared to macro conidia (Fig 1).

Pathogenicity Test and Symptomatology of *F. oxysporum* f.sp. *Lycopersici*

Pathogenicity study for the soil borne pathogen, *F. oxysporum* f.sp. *lycopersici* showed that the symptoms were expressed after 7 days of inoculation in tomato variety PKM-1. The symptoms were characterized as the first appearance of slight vein clearing on the outer portion of the younger leaves and followed by epinasty of the older leaves followed by stunting, yellowing of the lower leaves, formation of adventious roots, wilting of leaves and young stems, defoliation, marginal necrosis of remaining leaves and finally death of the entire plant. The pathogen was identified as virulent.

Screening of *Pseudomonas Fluorescens* Strains Against the Pathogen Under *in Vitro*

Fifteen isolates of *P. fluorescens* were tested for their efficacy by dual plate technique against *F. oxysporum* f.sp. *lycoopersici*. All the strains were significantly



Pathogen growth on PDA medium **Macro and Micro conidia of the pathogen**

Figure 1: Cultural and morphological characters of *F.oxysporum* f.sp. *lycopersici*

Table 2
In vitro*screening of *P. fluorescens* strains against *F.oxysporum* f.sp. *lycopersici

S. No.	*Isolate code*	*Mycelial growth of the pathogen (mm)*	*Per cent inhibition over control*
1.	Pf 1	55.40	38.44
2.	Pf2	62.30	30.77
3.	Pf3	70.00	22.22
4.	Pf4	54.00	40.00
5.	Pf5	57.00	36.66
6.	Pf 6	48.30	46.33
7.	Pf7	56.40	37.33
8.	Pf8	50.00	44.44
9.	Pf9	68.10	24.33
10.	Pf10	55.00	38.88
11.	Pf11	45.00	50.00
12.	Pf12	70.90	21.22
13.	Pf13	65.00	27.77
14.	Pf 14	63.00	30.00
15.	Pf 15	60.10	33.22
16.	Control	90.00	–

Values are means of three replications.
Means followed by a common letter are not significantly different at 1% level by DMRT.

inhibited the growth of the pathogen when compared to control. Among the fifteen strains, five strains *viz*., Pf 12, Pf 3, Pf 9, Pf 13 and Pf 14 were found to be more inhibitory to *F.oxysporum* growth when compared to other strains under *in vitro*. The *Pseudomonas* strain Pf 12 isolate showed highest inhibition to *F.oxysporum* f.sp. *lycoopersici* followed by Pf 3 (Table 2).

DISCUSSION

The indiscriminative use of fungicides leads to health hazards, environmental pollution and toxicity. It also reduces the beneficial microorganisms. Thus, it becomes necessary for finding alternative resources to reduce the chemical fungicides. The present day global interest in control of plant pathogens by biocontrol agents has a direct impact on economic assistance to farmers. There have been many research efforts to demonstrate the significance of *Pseudomonas* bacteria in the rhizosphere and on the roots.

In the present study, *F. oxysporum* f.sp. *lycopersici* was isolated from infected roots of tomato. The pathogen was multiplied in sand maize media and was used as inoculum source. The pathogenicity of isolated *F. oxysporum* f.sp. *lycopersici* was proved by reisolating from artificially infected tomato plants. This study was supported by Ramamoorthy *et al*. (2002) Kalpana (2008) and Manikandan *et al.* (2010), they reported that *F. oxysporum* f.sp. *lycopersici* produces, browning of the vascular tissue is one of the characteristic symptoms of *Fusarium* wilt. Further, on older plants, symptoms generally become more apparent during the period between blossoming and fruit maturation. The pathogenicity was proved as per the standard method and the pathogen was found to be more virulent.

Fifteen isolates of *Pseudomonas* were tested for antagonistic effect against *F. oxysporum* f.sp. *lycopersici.* Among them Pf 12 was found best in reducing the mycelia growth of *F.oxysporum* f.sp. *lycopersici* under *in vitro*. Similar results was supported by Ramamoorthy *et al.* (2002) who reported that, *Pseudomonas* strain Pf1 showed higher inhibition when tested against tomato *Fusarium* wilt. Sundaramoorthy (2012) reported that compared to individual treatment of bacterial strain, combination of EPCO16+EPC5+Pf1 was found best in reducing the mycelia growth of *Fusarium solani*. Kalpana (2008) reported that biocontrol strains exhibited high level of inhibition against *F. oxysporum* f.sp. *lycopersici. Pseduomonas* strains significantly reduced the mycelial growth of *F. oxysporum* f.sp. *lycopersici* under *in vitro* (Manikandan *et al*., 2010; Shanmugam and Kanoujia, 2011; Mohammed Faisal *et al*., 2014a). In conclusion, among various strains of *P. fluorescens*, Pf3 has the potential to suppress growth of *F. oxysporum* f.sp. *lycopersici* and could be used as a potential biocontrol agent against the management of wilt disease in tomato.

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