IMPACT OF RHIZOSPHERE *PSEUDOMONAS FLUORESCENCE* AGAINST *ALTERNARIA SOLANI* IN TOMATO

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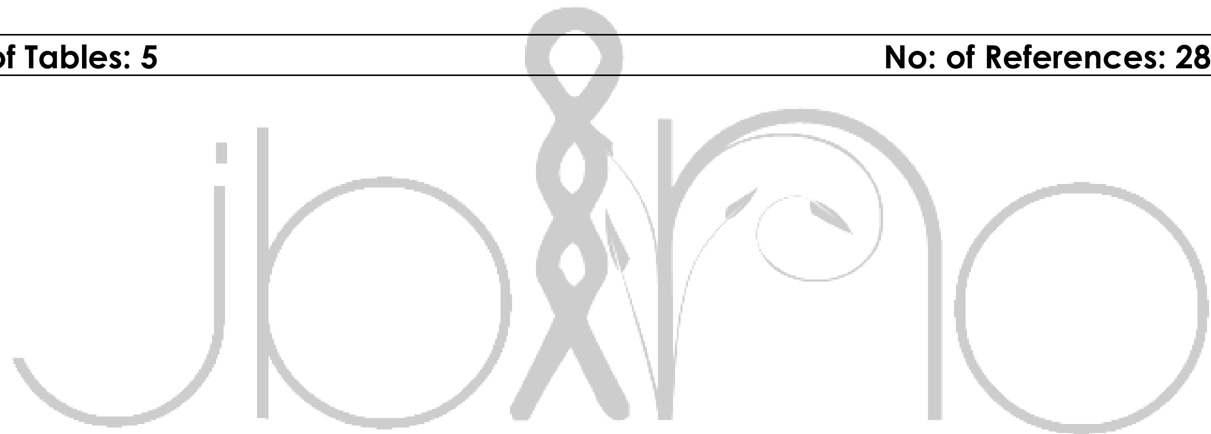
(Received on Date: 15 January 2018 Date of Acceptance: 28 February 2018)

Abstract

Dual culture technique indicated that all the isolates inhibited the growth of test fungus significantly. Among the isolates, Pf_3 produced minimum mycelia growth (10.21mm) accounting for 88.65 per cent reduction over control. The isolates Pf_3 recorded the maximum germination percentage (93.80%), shoot length (11.50 cm), root length (12.95 cm) and vigour index (2293.41).

Keywords: Tomato, *Alternaria solani, Pseudomonas fluorescence*

No: of Tables: 5 **No: of References: 28**



INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) belongs to the family solanaceae is one of the most remunerable and widely grown vegetable in the world. It is cultivated for its fleshy fruit and the area under its cultivation is increasing day by day due to its nutritional value, demand and high yield. Tomato is a regular kitchen component of Indian diet which is cooked in the form of various processed products like juice, ketchup, sauce, pickle, pastes and powder. It also has high medicinal value i.e the pulp and juice is digestible, promotes gastric secretion and purifies blood. It is an excellent source of folate, potassium, vitamin A and C as well as lycopene – a natural antioxidant, which is not found in any other solanaceous crops (Anonymous, 2008). It is grown in an area of 22,433 ha, with a production of 2, 82,912 tonnes with a productivity of 12,611 kg/ha in Tamil Nadu (Anonymous, 2013).

India is the fourth largest producer of tomato globally, contributing around 11.9 MT/ year. However, its average production is lesser (19.6 MT/hac) compared to the world average (28.2 MT) (Anonymous, 2014). The yield of tomato is restricted to a great extent due to different diseases and insect pests associated of these, tomato is highly most vulnerable to early blight, late blight and *Fusarium* wilt (Panthee and Chen, 2010). Early blight infection cause deterioration in the quality of tomato, minimize yield and wich find less market value.

In India, the yield loss due to this malady was estimated 10 to 80 per cent (Singh, 1985; Abada *et al*., 2008). Early blight of tomato is mainly managed by chemicals (Singh *et al*., 2001). At the same time indiscriminate use of chemicals caused the development of fungicidal resistance by the pathogen, environmental pollution and health hazards (Rai *et al.,* 2000).Therefore, an alternative strategy for the management of this malady is the need of hour considering the importance of disease, *Pseudomonas fluorescens* is evaluated against *Alternaria solani* under laboratory conditions.

MATERIALS AND METHODS

Isolation of *Pseudomonas fluorescens*

Rhizoplane - colonizing *P. fluorescens* was isolated from fresh roots of tomato grown in 6 different regions of cuddalore district. The soil particles loosely adhering to the roots were tested out and used for the isolation of *P fluorescens*. A soil suspension was prepared from each rhizophere sample by shaking one g of soil sample in 10ml of sterile dist. water and serial dilutions were made. One ml of soil suspension from aliquot dilutions (10^{-5} to 10^{-8}) was aseptically added to sterile Petri dishes containing twenty ml of sterile King's medium and incubated at 28±2°C for 48 h. after incubation, well separated individual colonies with yellow green and blue white pigments were marked and detected by viewing under UV light. The individual colonies were picked up with sterile loop and transferred to fresh King's B slants and the pure cultures so obtained were stored in refrigerator at 4°C for further use.

Table 1. Isolation collection of plant growth promoting rhizobacterium

Isolates	Cell shape	Colony type	Colour of the colony	Reaction to UV light
Pf_1 (Bhuvanagri)	Rod	Irregular	Light greenish	Fluorescent
Pf_2 (Kumarachi)	Rod	Round	Light greenish	No reaction
Pf_3 (Neyveli)	Rod	Irregular	Light greenish	Fluorescent
Pf_4 (Lalabeat)	Rod	Round	Light greenish	Fluorescent
Pf_5 (Vadalure)	Rod	Irregular	Light greenish	No reaction
Pf_6 (Subramaniyapuram)	Rod	Round	Light greenish	Fluorescent

Mode of action by rhizosphere inhabiting *Pseudomonas fluorescens*

Estimation of IAA

Indole acetic acid (IAA) in the methanol fraction was determined by employing Salper reagent (Gordon and Paleg, 1975). To 1.5 ml of distilled water in a test tube 0.5 ml of methanol residue was mixed, four ml fresh Salper reagent was rapidly added, kept in complete darkness for one hour and read in spectrophotometer at 535 nm. From a standard curve prepared with known concentration of IAA, the quantity of IAA in the filtrate was calculated (1 division = 0.307 µg of IAA).

Extraction of siderophore from the medium

The spent culture fluid was separated from the cells by certifugation at 7000 rpm for 15 min. The supernatant was concentrated to one fifth of the original volume by flash evaporation at 45°C. Catechol type phenolates were extracted with ethyl acetate from the culture supernatant twice with an equal volume of solvent at pH 2.0. The ethyl acetate layer was removed and evaporated to dryness and the residues were dissolved in a minimum quantity of dist. water, while hydroxamate types were measured from the untreated culture supernatant (Schwyn and Neilands, 1987).

Hydrogen Cyanide (HCN) production

Production of HCN was determined as per the method of Wei *et al.*, (1996). Bacteria were grown on TSA supplemented with 4.4g/1 of glycine. White filter paper strips soaked in picric acid solution (2.5 g of Na_2CO_3 and 1 litre of water) were placed in the lid of each Petri dish, sealed with parafilm and incubated for two to three days at 28±2°C. After incubation HCN production was indicated by the presence of a coloured zone around the bacteria.

Dual culture technique

The antagonistic activity of *P fluorescens* against *A.solani* was

evaluated using dual culture technique (Dennis and Webster, 1971). *P. fluorescens* isolate, Pf1 was isolated from the rhizosphere of rice ecosystem, which was obtained from the Department of Plant Pathology, TNAU.*P fluorescens* was streaked at one side of Petri plate containing PDA. A 9 mm mycelial disc from seven days old potato dextrose agar (PDA) culture of *A. solani* was placed in the opposite direction *P. fluorescens* of and incubated at 28±2°C for 15 days. Due to slow growth of *A.solani P. fluorescens* was inoculated after 72 h of inoculation of the pathogen in petri plate. Petri dishes inoculated with fungal discs alone served as control. Each treatment was replicated thrice. Observation regarding inhibition zone and mycelial growth of pathogen was recorded. The per cent inhibition of pathogen growth was calculated using the formula of Vincent (1927) i.e,.

$$I = C-T/Cx100$$

Where,

I- Per cent inhibition

C-Mycelial growth of pathogen in control

T- Mycelial growth of pathogen in treatment

Poisoned food technique

The culture filtrate of *P. fluroescens* was separately incorporated into sterilized PDA medium @ 5,10, 15 and 20 per cent using different quantity of culture filtrate. The uninoculated petridishes served as control. The mixed media was transferred in each petri dishes @ 15 ml/petridish. The each pertidish allowed to solidify then *A.solani* innoculated in the centre of each petridish and incubated at (28±2°C) for 15 days. Mancozeb 75% WP @ 0.25% used as chemical control. Each treatment replicate thrice. Observation with respect to radial growth of mycelium were taken after 15 days of incubation period. Finally per cent inhibition over control was recorded

Plant growth promotion

To study PGPR in tomato, inoculums of *P. fluorescens* was prepared by following the method of Thomson, 1996. After preparation of inoculums, it was applied by seed treatment and Roll Towel method (ISTA, 1993). Finally the observations on seedling vigour index was calculated as mentioned by Abdual Baki and Anderson (1973).

Vigour index (VI) = (Mean root length + Mean shoot length) x Germination (%)

Result and discussion

The result of the dual culture technique indicated that all the isolates inhibited the growth of test fungus significantly. Among the isolates, Pf_3. produced maximum reduction of mycelia growth (10.21mm) accounting for 88.65 per cent reduction over control. This was followed by the isolates Pf_4, Pf_6, Pf_2 Pf_5 and Pf_1 recording (86.14, 85.53, 83.21, 78.04, 74. 95 per cent). The isolates Pf_1 was the least effective recording 74.95 per cent inhibition over control.

In the present study, all the six native isolates of *P. fluorescens* showed varying degrees of antagonism against *A. solani*. Among the isolates, Pf_3 was the most antagonistic and formed the maximum inhibition zone and maximum per cent reduction on the mycelial growth of *A. solani*.

The mycoparasitic potential of *Pseudomonas* spp. is well documented (Whipps, 1997) and this phenomenon has often been used as means for *in vitro* screening of biocontrol agents (Elad *et al*., 1980). *P. fluroescens* isolates EBS 20 produced higher levels of extracellular metabolites like siderophore, salicylic acid and HCN when compared with other isolates which was highly effective in inhibiting the growth of *Pythium aphanidermatum* inciting chilli damping-off. (Muthukumar *et al*., 2010a). Similarly, antifungal compounds such as pseudobactin, HCN, salicylic acid and 2-hydroxy phenazine produced by fluorescent pseudomonas suppressed plant pathogenic fungi (Pandey *et al*., 2006; Reddy *et al*., 2008). The antifungal metabolites produced by *P. fluorescens* might be attributed as the reason for the reduction in the growth of the pathogen and *P. fluorescens* were known to produce an array of low-molecular weight metabolites some of which were potential antifungal agents (O' Dowling and O' Gara, 1994). Earlier workers reported that *P. fluorescens* effectively reduced mycelial growth of other pathogens (Sundaramoorthy and Balabasker, 2012; Meera and Balabaskar, 2012a; Nandi *et al*., 2013; Meera, 2013; Sundaramoorthy, *et al*., 2013).

The results presented in revealed that all the isolates showed reduction on mycelial growth of *A.solani*. Among the isolates, Pf_3 at concentration of 5, 10, 15 and 20 per cent concentration showed an increase in the inhibition of the mycelial growth recording (15.28, 13.57, 9.43 and 5.12) respectively. Also, a general increase in the concentration of the culture filtrate showed an increase in the inhibition of the mycelial growth of the test pathogen. Among the isolates the culture filtrate of Pf_3 at 20 % conc. was found to be effective in reducing the mycelial growth to the minimum (5.12mm) accounting for the highest per cent inhibition (94.31%) of the pathogen over control. This was followed by Pf_4 @ 20% conc. recording (89.47% inhibition) and Pf_6 (84.01% inhibition). The least effect was found with isolate Pf_1 (80.38 per cent inhibition)

Efficacy of PGPR's on seed germination and plant growth promotion

The data presented in table 3 revealed that the isolates of *P. fluroescens* showed an increase in the germination of tomato seeds and induced the plant growth significantly, when compared to control. However, among the six isolates, the isolate Pf_3 recorded the maximum germination per cent (93.80 per cent), shoot length (11.50 cm), root length (12.95 cm) and vigour index (2293.41). This was followed by Pf_4, Pf_6, Pf_2, Pf_5, and Pf_1 in the decreasing order of merit. The untreated control recorded the lowest values in terms of germination per cent (76.00%), shoot length (7.5 cm), root length (8.01cm) and vigour index (1178.76).

In the present study, *P. fluorescence* all the isolates of *P. fluorescence* increased the germination of tomato seeds and induced the plant growth promotion and yield of tomato crop. The studies on the mechanism of growth promotion indicates that PGPR promote plant growth directly by production of plant growth regulators (Idriss *et al*., 2002) or indirectly by stimulating nutrient uptake, by producing

siderophores or antibiotics to protect plant from soil borne pathogens or deleterious rhizosphere organisms (Dobbelaere *et al*., 2003). *Pseudomonas* spp. may increase plant growth by producing gibberellin-like substance (Brown, 1972), mineralizing phosphates (Kavimandan and Gaur, 1971). The growth promoting substances produced by *P. fluorescens* might have exerted a synergistic action and enhance the growth promotion of tomato. Idriss *et al*., (2002) observed phosphate solubilizing bacteria positive for IAA, gibberellin and cytokinin production. Manikandan *et al*., (2010) reported *P. fluorescens* Pf1 had the ability to significantly increase the vigour index of tomato seedlings. All these earlier reports lend support to the present findings

Conclusion :

In the *invitro* analysis,native isolates of *P. fluorescens* showed antagonism against *A. solani*., Pf_3 was the most antagonistic and formed the maximum inhibition zone and maximum per cent reduction on the mycelial growth of *A. solani*. The mechanism of growth promotion indicates that PGPR(Pf_3) promote plant growth directly by production of plant growth regulators and for the management of early blight incidence under field condition

Table 2. Mode of action by rhizosphere inhabiting bacteria

Isolates	Estimation of IAA (µg/ml)	Siderophore production (Hydroxamate) (µg/ml)	Hydrogen cyanide production (µg/ml)
Pf_1	2.3^f	0.54^e	6.30^f
Pf_2	2.9^d	0.79^c	7.69^d
Pf_3	3.8^a	0.86^a	8.20^a
Pf_4	3.5^b	0.84^b	7.99^b
Pf_5	2.4^e	0.55^d	6.42^e
Pf_6	3.3^c	0.84^b	7.86^c

Values in the column followed by same letters not differ significantly by DMRT (p=0.05); figures in the parentheses represent arc sine transfermed values

Table3. Efficacy *P. fluorescens* against *A. solani in vitro* using dual culture technique

Native isolates	*A. solani*	
	Mycelial growth the pathogen (mm)	Per cent inhibition over control (%)

Pf$_{1}$	22.54^{f}	74.95 (59.93)
Pf$_{2}$	15.11^{d}	83.21 (65.80)
Pf$_{3}$	10.21^{a}	88.65 (70.27)
Pf$_{4}$	12.47^{b}	86.14 (68.11)
Pf$_{5}$	19.76^{e}	78.04 (62.03)
Pf$_{6}$	13.02^{c}	85.53 (67.62)
Control	90.0	00.00

Values in the column followed by same letters not differ significantly by DMRT (p=0.05); figures in the parentheses represent arc sine transfermed values

Table 4. Effect of culture filtrates of *P. fluorescens* against *A. Solani* using (Poisoned food technique).

S. No	Isolates	Mycelia growth (mm)				Per cent inhibition over control			
		5	10	15	20	5	10	15	20
1.	Pf$_{1}$	20.61^{f}	19.23^{g}	18.43^{f}	17.65^{g}	77.10^{g} (61.34)	78.63^{g} (62.44)	79.52^{e} (63.08)	80.38^{f} (63.65)
2.	Pf$_{2}$	18.59^{e}	17.03^{e}	16.14^{e}	15.84^{e}	79.34^{e} (62.94)	81.07^{e} (64.16)	82.06^{d} (64.90)	82.40^{e} (65.20)
3.	Pf$_{3}$	15.28^{b}	13.57^{b}	9.43^{b}	5.12^{b}	83.02^{b} (65.65)	84.92^{b} (67.13)	89.52^{b} (71.09)	94.31^{b} (76.18)
4.	Pf$_{4}$	16.21^{c}	15.01^{c}	13.39^{c}	9.47^{c}	81.98^{c} (64.82)	83.32^{c} (65.88)	85.12^{c} (67.29)	89.47^{c} (71.00)
5.	Pf$_{5}$	19.41^{e}	17.84^{f}	16.01^{e}	16.47^{f}	78.43^{f} (62.31)	80.17^{f} (63.51)	82.21^{d} (65.05)	81.70^{e} (64.67)
6.	Pf$_{6}$	17.20^{d}	16.12^{d}	15.31^{d}	14.39^{d}	80.88^{d} (64.01)	82.08^{d} (64.90)	82.98^{d} (65.57)	84.01^{d} (66.42)
7.	Mancozeb 75%	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	100^{a}	100^{a}	100^{a}	100^{a}

	WP (0.25% conc.)								
8.	Control	90[g]	90[h]	90[g]	90[h]	0.00[h]	0.00[h]	0.00[f]	0.00[g]

Values in the column followed by same letters not differ significantly by DMRT (p=0.05); figures in the parentheses represent arc sine transfermed values

Table 5. Effect of *P. fluorescens* on different plant growth parameters in tomato seedling (Roll towel method)

S. No	Isolates	Seed germination (%)	Shoot length (cm)	Root length (cm)	Vigour index
1.	Pf_1	88.50[d] (70.18)	9.00[d]	9.55[d]	1641.67[d]
2.	Pf_2	89.50[c] (71.09)	10.70[c]	10.12[c]	1863.39[c]
3.	Pf_3	93.80[a] (75.58)	11.50[a]	12.95[a]	2293.41[a]
4.	Pf_4	86.00[f] (68.03)	11.00[f]	11.65[f]	1947.90[f]
5.	Pf_5	87.00[e] (68.87)	10.15[e]	10.21[e]	1771.32[e]
6.	Pf_6	90.50[b] (72.05)	10.98[b]	9.86[b]	1886.02[b]
7.	Control	76.00[g] (60.67)	7.50[g]	8.01[g]	1178.76[g]

Values in the column followed by same letters not differ significantly by DMRT (p=0.05); figures in the parentheses represent arc sine transfermed values

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