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Potentialities of *Pseudomonas fluorescens* for Management of Fusarium Wilt Disease of Tomato in Central Himalayas

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A B S T R A C T

Pseudomonads are well known for their growth promotion activity and defense induction against the phytopathogens in plants leading to the disease incidence delay. Effect on the disease incidence with the interaction of different strains of *Pseudomonas fluorescense* were studied in tomato under protected and open conditions. The strains were isolated from different soil samples collected from various geographical regions of central Himalaya. Promising strains of Pseudomonas were applied as seed bio priming (SB), SB+ Root Dip (RD), SB+RD+ Drenching (DR) and SB+RD+DR+ Spraying (SPR) individually and in combination with different strains and evaluated for their effect on management of wilt caused by *Fusarium oxysporum* f. sp. lycopersici, an important disease of tomato in central Himalaya. Seeds were bio- primed with bio-formulated products RS-01, PS-01, AS-01, and consortia of different strains RC-05, AC-05, and PC-05 before sowing. Amongst the bio- products used AC-05 was found most effective in reducing the wilt incidence.

Introduction

Biological control can be defined as the use of natural enemies to reduce the damage by a pest population. It is an approach that fits into an overall pest management programme, and represents an alternative to continued reliance on pesticides (Cook and Baker, 1983). It has been used as a management tool for control of crop and forest pests, and for the restoration of natural systems affected by adventive pests. Biological control in the broadest sense could be defined as the use of living agents to control plant pathogens. Over the past one hundred years, research has repeatedly demonstrated that phylogenetically diverse microorganisms can act as natural antagonists of various plant pathogens (Cook, 2000). Bacterial inoculants, widely used as bio-control agents, are applied to soil, seed or roots of agriculture crops for the suppression of soil born plant diseases. Rhizosphere bacteria with the ability to provide biological control appear to comprise less than 10% of the total population of bacteria in the rhizosphere (Weller, 1988). Specific

rhizobacteria associated with plant roots and stimulate plant growth promoting rhizobacteria (PGPR) (Kloepper and Schroth, 1978). PGPRs induce resistance in plants and suppress plant pathogens causing fungal, bacterial and viral diseases.

Tomato (*Lycopersicon esculantum* Mill) is an important vegetable crop grown throughout the word moreover as a cash crop (Tewari and Vishunavat, 2012). Diseases are most limiting factors for production of tomato leading to a reduction in its quantity and market value. The major fungal diseases of tomato are early blight, late blight, damping off, root rot, buckey rot (fruit rot), fusarium wilt, collar rot, powdery mildew, white rot, gray leaf spot, verticillium wilt, anthracnose and black leaf mould. Wilt caused by *Fusarium oxysporum* pv. lycopersici is an important disease in central Himalaya, which may have devastating effects on economy of farming community, if the disease is of epidemic nature and not under control (Whipps and Lumsden, 1991). The causal organism is of soil – borne in nature and difficult to control, thus making the development of alternatives a high priority (Lemanceau *et al.*, 1992). Pseudomonads spp. are well known to show positive interaction with microorganisms resulting in growth promotion in plants and simultaneously activating the disease incidence delay by synthesizing antibodies and growth promoting substance. Keeping in view the efficacy of *Pseudomonads* spp as PGPR and as an antagonist, the present study was conducted to investigate these for prospective management of Fusarium wilt disease of tomato in Central Himalayan regions.

Materials and Methods

Isolation and identification of *Pseudomonads*

Different soil samples having pH values ranging from 5.75 to 6.77 were collected from diverse agro climatic locations of central Himalayan regions of Uttarakhand viz., Auli (9000'), Pithoragarh (5500') and Raiwala (942'). Susceptible cultivars of tomato were grown in pots filled with these soil samples. The plants were gently uprooted with approximately 10cm diameter core of surrounding soil with least possible injury to the roots. Root system and surrounding soil were placed in plastic bags and transported back to the laboratory in an ice chest. After vigorous shaking of excited roots to remove tightly adhering soil, 1g of soil was mixed with 9ml sterilized distilled water under aseptic conditions to make the dilution 10-1. This was further diluted up to 10-7 dilutions. The rhizospheric suspensions were plated (1ml) on Kings' B medium (KB) (King et al; 1954) by streaking method. After 48 h. of growth at 28±1ºC, dominant fluorescent colonies were viewed under UV light and selected on the basis of colony characters and morphology of the bacteria and subjected to single colony isolation. The bacterial strains then selected were sent to Institute of Microbial Technology (I M Tech) Chandigarh for identification as well as transferred on the Kings' B slants and kept at 4ºC for further studies. Pure culture of test pathogen *Fusarium oxysporum* was initially collected from IARI Delhi.

Talc based formulation of effective strains of Pseudomonas were prepared by mixing autoclaved Carboxy methyl cellulose (CMC) and talc powder @ 1% w/w with bacterial strains grown on KB broth for 48 hours in incubator shaker at 150 rpm and 27±1°C for 24 hours on each of two consecutive days. 500ml of the bacterial suspension containing 2X109 colony forming units (cfu) per ml was added to one kg of the carrier, mixed thoroughly and left overnight for complete drying. For the preparation of mixed formulation 100ml broth of each bacterial suspension was mixed in one kg carrier.

Screening of Fluorescent *Pseudomonas* against *F. oxysporum* through in vitro antagonism

The antagonistic activity of different strains of Pseudomonas against the pathogenic fungi was assessed on agar plates as described by Weller *et al.,* (1988) with slight modifications. A combination of two media (KB+PDA 1:1) was used for this purpose. Pure culture of bacterial strains was grown on Kings' B broth and fungal pathogen maintained on PDA slants was transferred to Petri dishes containing fresh PDA to produce fungal mycelium plugs. Petriplates were inoculated with the help of sterilized glass bangle and a 5 mm mycelial disc (from actively growing colony of the test pathogen) was cut with the help of sterilized cork borer and placed in the centre of Petri dish. These inoculated plates were incubated at 28±1ºC. Maximum and minimum inhibition zones were measured after 48, 72, 96 hours. The plates without bacteria served as control. Three replications were taken for each treatment.

Assessment of disease suppression potential of Pseudomonads

Seeds of tomato var. Punjab Chhuhara were surface sterilized with 2 per cent sodium hypochloride for 30 seconds and rinsed in sterilized distilled water and dried overnight. Sterilized seeds were treated with 10 per cent solution of bio formulations for 30 minutes and then dried in sterile airflow overnight. Treated seeds were planted into the pots (60X45cmX10cm Size), and field under glass house conditions. Three replications were made for each treatment and observations with respect to disease incidence were recorded. To record the performance of various treatments, during crop growth stage, transplanting was done in pots as well as in field under glass house. Bio formulation treatments were given as (a) Seed bio-priming + root dip (SB+RD) (b) SB+RD+Dreanching (SB+RD+DR) (c) SB+RD+DR+ foliar spray (SB+RD+DR+SPR). Drenching and root dip @ 10 per cent whereas foliar spray @ 5 per cent bio formulation was done. Experiments were set in triplicate, in randomized arrangement. Observations with respect to disease incidence at seedling and crop stage were recorded for two crop seasons.

Results and Discussion

Isolation and identification Fluorescent pseudomonads

Eighty six strains of Pseudomonads were collected from different regions of central Himalayas. The isolated cultures were send for identification to Institute of Microbial Technology (IMTech) Chandigarh, and were identified as different strains of Fluorescent pseudomonas, on the basis of morphological and chemical characters. Pure cultures of pathogenic fungi, *Fusarium oxisporum* causing wilt disease of tomato in central Himalayan region was brought from IARI Delhi. These cultures were used for antagonistic activity assessment as well as for inoculation of tomato plants. The plants inoculated with the cultures showed wilting symptoms.

Symptomatology

Fusarium oxysporum and its various forms have been characterized as causing the following symptoms viz. vascular wilt, yellows, corm rot, root rot, and damping-off. Fusarium wilts first appear as slight vein clearing on the outer portion of the younger leaves, followed by epinasty (downward drooping) of the older leaves. At seedling stage, plants showed wilting and death soon after symptoms appearance. In older plants, vein clearing and leaf epinasty often followed by stunting, yellowing of the lower leaves,

formation of adventitious roots, wilting of leaves and young stems, defoliation, marginal necrosis of remaining leaves, and finally death of the entire plant was observed.

Browning of the vascular tissue is strong evidence of Fusarium wilt. Further, on older plants, symptoms generally become more apparent during the period between blossoming and fruit maturation (Altinok, 2005).

In vitro screening of Fluorescent Pseudomonads against F. oxysporum

Pseudomonads when screened against *Fusarium oxisporum*, the strains isolated from the soil samples collected from Auli (AULI-200, AULI-178, AULI-185, AULI-188, AULI-187) were most antagonistic followed by those from the soil samples of Pithoragarh (P GGu-667, P JB-669, P JB-531, P GI-446, P JB-662) and Raiwala (R RWL-128, R RWL-308, R GRW-329, R GRW-118, R RWL-330) where maximum zone of inhibition was recorded. The Auli strains isolated from the higher hills were comparatively more effective than that of lower hills.

In vivo screening through seed bio-priming

The effect of formulated products on disease incidence was studied in tomato variety Punjab Chhuhara. Seeds were bio- primed with bio-formulated products RS-01 (strain R RWL-128), PS-01 (strain P GGu-667), AS-01 (strain AULI-185) and consortia of different strains RC-05 (consortia of R RWL-128, R RWL-308, R GRW-329, R GRW-118, R RWL-330), AC-05 (consortia of AULI-200, AULI-178, AULI-185, AULI-188, AULI-187), and PC-05 (consortia of P GGu-667, P JB-669, P JB-531, P GI-446, P JB-662) before sowing. Formulated products which were prepared by mixing five effective strains showed higher efficacy towards disease suppression as compared to those prepared using single bacterial strain. Amongst the bio-products used AC-05 was found most effective in reducing the wilt incidence as minimum wilting 3.7 per cent, 5.3 per cent, 10.7 per cent and 11.3 per cent wilting was recorded after 10, 15, 20 and 25 days after germination respectively in pot conditions. Bio-inoculant AC-05 was also found highly significant in reducing the wilting under field conditions (Table 1).

Table.1 Progressive disease incidence at seedling stage in pots and field under protected conditions

Treatment	Disease incidence (%)							
	Pot conditions				**Field conditions**			
	Days after Germination				**Days after Germination**			
	10	15	20	25	10	15	20	**25**
RS-01	16.3	18.3	24.7	25.3	16.0	20.0	29.0	**30.0**
PS-01	11.7	14.7	20.3	22.3	15.0	18.0	26.0	**28.0**
AS-01	5.3	8.3	12.6	15.3	08.0	10.0	18.0	**19.0**
RC-05	10.3	12.3	14.3	17.3	12.0	15.0	22.0	**24.0**
AC-05	3.7	5.3	10.7	11.3	5.0	07.0	12.0	**13.0**
PC-05	5.3	6.3	11.3	12.3	6.0	08.0	14.0	**16.0**
Control	25.0	27.3	36.6	40.0	30.0	34.0	48.0	**53.0**
CD at 5 %	20.376	17.130	10.390	8.212	20.104	14.173	08.234	**08.833**
CV	**1.305**	**1.309**	**1.119**	**0.979**	**1.578**	**1.309**	**1.147**	**1.333**

Table.2 Progressive disease incidence at different crop stages in pots under protected conditions

Treatments	Disease incidence (%)														
	15 DAT			30 DAT			45 DAT			60 DAT			**90 DAT**		
	SB+RD	SB+RD +DR	SB+RD+ DR+SPR	SB+RD	SB+RD +DR	SB+RD+ DR+SPR	SB+RD	SB+RD +DR	SB+RD+ DR+SPR	SB+RD	SB+RD +DR	SB+RD+ DR+SPR	SB+RD	SB+RD +DR	**SB+RD+ DR+SPR**
AC-05	9.14	5.29	7.84	11.06	9.66	12.00	35.58	30.73	24.27	48.89	37.15	30.25	59.10	44.52	**41.41**
RC-05	15.77	12.27	12.81	28.22	25.24	25.04	53.24	39.10	26.90	71.42	50.32	43.67	89.25	68.45	**59.17**
PC-05	10.42	8.61	9.37	19.20	17.37	21.38	50.19	39.88	27.70	68.03	47.33	43.21	83.68	77.09	**52.53**
Control	18.53			34.42			62.81			77.53			**93.85**		
CD at 5 %	(a)=.838 (b)=.967 (a*b)=1.676			(a)=1.286 (b)=1.485 (a*b)=2.573			(a)=2.118 (b)=2.446 (a*b)=4.237			(a)=4094 (b)=4.727(a*b)=8.188			**(a)=1.642 (b)=1.896 (a*b)=3.285**		
CV	**8.072**			**6.693**			**5.819**			**8.753**			**2.717**		

Table.3 Progressive disease incidence at different crop stages in fields under protected conditions

Treatments	Disease incidence (%)														
	15 DAT			30 DAT			45 DAT			60 DAT			**90 DAT**		
	SB+RD	SB+RD +DR	SB+RD+ DR+SPR	SB+RD	SB+RD +DR	SB+RD+ DR+SPR	SB+RD	SB+RD +DR	SB+RD+ DR+SPR	SB+RD	SB+RD +DR	SB+RD- DR+SPR	SB+RD	SB+RD +DR	SB+RD+ DR+SPR
AC-05	7.81	2.76	5.81	9.64	10.57	6.73	31.69	24.63	17.73	42.62	33.62	27.59	58.18	43.67	39.67
RC-05	9.38	9.84	9.90	22.33	20.49	21.53	50.52	37.57	22.12	64.50	42.49	40.07	83.07	66.59	57.41
PC-05	9.76	7.15	7.34	15.70	18.72	14.16	42.26	32.37	21.52	54.96	48.31	35.07	77.42	73.47	49.36
Control	18.34			29.75			59.00			72.81			88.32		
CD at 5 %	(a)=1.127 (b)=1.302 (a*b)=2.255			(a)=0.943 (b)=1.088 (a*b)=1.886			(a)=1.085 (b)=1.253 (a*b)=2.170			(a)=1.326 (b)=1.531 (a*b)=2.653			(a)=1.171(b)=1.352 (a*b)=2.342		
CV	**12.803**			**05.832**			**03.362**			**03.094**			**02.039**		

Table.4 Progressive disease incidence at different crop stages in open field conditions

Treatments	Disease incidence (%)														
	15 DAYS			30 DAYS			45 DAYS			60 DAYS			**90 DAYS**		
	SB+RD	SB+RD +DR	SB+RD+ DR+SPR	SB+RD	SB+RD +DR	SB+RD+ DR+SPR	SB+RD	SB+RD +DR	SB+RD+ DR+SPR	SB+RD	SB+RD +DR	SB+RD+ DR+SPR	SB+RD	SB+RD +DR	**SB+RD+ DR+SPR**
AC-05	7.05	2.31	5.41	6.82	9.02	5.19	30.02	22.08	14.57	40.10	30.31	25.68	56.14	40.71	**38.69**
RC-05	11.14	9.11	9.78	18.99	20.07	18.41	48.43	36.55	20.49	62.75	41.60	39.75	72.23	62.00	**56.63**
PC-05	8.48	5.44	7.05	14.00	16.05	12.00	40.13	30.89	18.13	51.90	45.84	30.83	70.73	69.78	**42.83**
Control	14.05			24.96			56.68			70.77			**80.93**		
CD at 5 %	(a)=.491 (b)=.567 (a*b)=.982			(a)=.263 (b)=.304 (a*b)=.527			(a)=.331 (b)=.382 (a*b)=.662			(a)=.375 (b)=.433 (a*b)=.750			**(a)=.351 (b)=.405 (a*b)=.702**		
CV	**6.452**			**1.913**			**1.088**			**0.915**			**0.661**		

All other formulations when bio-primed in seeds showed significantly effective outcome in reducing the wilting of seedlings as compare to control. Suppression of plant pathogenic fungi and production of antifungal compounds by Pseudomonas spp. is also documented (Anitha and Tripathi, 2001; Bhowmik *et al.,* 2002; Gupta *et al.*, 2001). Pseudomonas fluorescens could act as strong elicitors of plant defense reactions (Piga *et al.*, 1997).

Effect of fluorescent Pseudomonads on disease suppression at different crop stages

Bio-formulated products RC-05, AC-05 and PC-05 prepared as consortia of different strains which were found effective at seedling stage were further assessed for their affectivity towards disease suppression at different crop stages after transplanting. Different sets of application methods as seed bio-priming (SB), root dip (RD), drenching (DR) and foliar spray (SPR) were followed and all the treatments in either of the application method were found effective as compared to control in both protected and open field conditions.

Under protected conditions, when seedlings were transplanted in pots as well as in field, minimum disease incidence was observed in AC-05 than other formulations in different crop stages up to 90 days of transplanting (DAT). However, all the formulations were found significantly effective than control in reducing the disease incidence (Table 2 and 3).

Among the methods of application, SB+RD+DR+SPR was found superior as disease incidence was observed least followed by SB+RD+DR and SB+RD. All the formulations were found significantly effective in managing the disease in different methods of applications. Similar trend of observations were also recorded in the open field conditions (Table 4). AC-05 product showed minimum wilting in different crop stages. However, during initial 30 DAT there was less difference among the bio-products treatments for disease management but after 90 days of transplanting significant difference was observed. The studies show that the wilt incidence has been delayed with different sets of treatments in different crop stages. It confirms that prior application of bio-inoculants in various combinations and different sets of applications, induces the plant's own defense mechanism which enhanced the production of defense related chemicals and enzymes in pants (Chenand Belanger *et al.*, 2000 and Masya *et al.*, 2010).

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