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Effect of *Pseudomonas fluorescens* in Controlling Bacterial Wilt of Tomato

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Abstract: Two variety of tomato viz. manik and pusha rubi were used to evaluate the efficacy of antagonistic *P. fluorescens* in controlling wilt of tomato caused by *R. solanacearum* and in increasing yield in green house condition. Four treatments i.e. *R. solanacearum* (10^6 c.f.u./ml) was used as control and *P. fluorescens* was used as 10^9, 10^8 and 10^7 c.f.u./ml suspension. Manik (V_1) showed least bacterial wilt incidence and pusa rubi (V_2) showed highest wilt incidence at 30, 45 and 60 DAI. T_1 (*P. fluorescens* 10^9 c.f.u./ml) and combined effect of V_1T_1 decreased wilt of tomato at all stages and increased fruit yield. T_0 (control-only *R. solanacearum* 10^6 c.f.u./ml) and V_2T_0 increased bacterial wilt at all stages and decreased fruit yield. So, T_1 and V_1T_1 may be used for controlling bacterial wilt of tomato and increasing yield.

Key words: Bacterial wilt, *Pseudomonas fluorescens*, *R. solanacearum*, tomato

Introduction

Tomato (*Lycopersicon esculentum* Mill) is an important popular vegetable of the world. It is popular because of its high nutritive value and diversified use (Bose and Som, 1986). Statistics show that in Bangladesh tomato was grown in 14,338 hectares of land and the total production was approximately 97,565 metric tones in 1998-99 with the average yield of 6.81 t/h (Anonymous, 1999a, 1999b) which is quite low compared to that of other tropical countries (Anonymous, 1999). Among the reasons, diseases caused by bacteria, fungi, virus and nematodes play a major role (Villaral, 1980). Bacterial wilt caused by *Ralstonia solanacearum* is one of the major bacterial diseases of tomato affecting its growth and yield. This disease can bring about almost total destruction of the crop during summer season. The loss of yield in tomato ranged from 10.83 to 90.60% while the plant mortality ranged from 10-100% (Ramkishun, 1987).

R. solanacearum has a wide host range. Soil is a potential source of primary inoculum and the disease has been noted even in first planting in newly cleared land (Kelman, 1953). So, cultural practice like crop rotation is not likely to be an effective or practical control method. Other control measures like host resistance has not yet become a viable control measure, because no resistant variety yet developed and released against this pathogen in Bangladesh. Neither cultural nor chemical measures were found to be effective against this pathogen. Biological control can be an alternative method. Biological control differs fundamentally from conventional chemical control of plant pathogens. Bio-control with antagonistic bacteria manipulates the environment around a crop plant to favour organism that contribute to increase fruit weight per plant (Gagne *et al.,* 1993). The bio-control is less destructive to ecosystem than that of chemical pesticides (Cook and Baker, 1983). Moreover plant growth promoting rhizobacteria (PGPR) isolated from rhizosphere and rhizoplane of different crops were found to be effective when they are co-inoculated with *R. solanacearum* (Amara *et al.*, 1996).

P. fluorescens is known antagonist of plant pathogenic bacteria and have been found to be very potential bio-control agent against soil borne plant pathogenic bacteria under both green house and field conditions (Anuratha and Gnanamanikam, 1990). *P. fluorescens* were found to be effective against *R. solanacearum* (Mulya *et al.,* 1996). Considering the above facts, biological control of bacterial wilt of tomato is needed. So, the present study has been undertaken to evaluate the efficacy of antagonistic *P. fluorescens* in controlling wilt of tomato caused by *R. solanacearum* and in increasing yield in green house condition.

Materials and Methods

The experiment was conducted at Agricultural Research Station, BARI, Bogra, Bangladesh during 2000–2001. Wilted tomato plants caused by *R. solanacearum* were collected from the farm of different places of Bogra district. Presence of pathogen was detected in the host by ooze test. The bacterium was isolated by extracting the ooze by the dilution plating technique. Well-separated virulent colonies of *R. solanacearum* were grown on nutrient agar (NA) media by streaking. Three to four loops full of the virulent colonies were suspended in sterilized distilled water taken in screw cap tubes. The tubes were stored at $\pm$ 5°C and considered as stock culture of the *R. solanacearum.* Pathogenicity test was carried out for the virulent culture of *R. solanacearum* isolated from diseased tomato plants.

P. fluorescens strain PF1 was collected from Bacteriology Division, Seed Pathology Laboratory, Bangladesh Agricultural University, Mymensingh. *P. fluorescens* isolate was purified on NA media and preserved in the screw cap tubes and considered as stock culture of the *P. fluorescens*. Twenty milliters sterile water was added in per plate culture of *P. fluorescens* and per plate culture of *R. solanacearum*. The bacterial colony was scrapped and mixed well with sterile water and it was used as stock suspension. *P. fluorescens* suspension contained 10^{10} c.f.u./ml and *R. solanacearum* suspension contained 10^7 c.f.u./ml.

Seeds of tomato var. manik (V_1) were collected from Horticulture Research Centre, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur and another var. pusa ruby (V_2) were collected from royal seed store, Natoon Bazar, Mymensingh, Bangladesh. Seeds of both cultivars were stored in refrigerator at 5-7°C until use for subsequent studies.

Soil was treated with formaldehyde as fumigant. The test soil was moistened with formaldehyde having concentration of 0.2% and kept under polythene cover for 7 days. The soil was then uncovered and allowed to be defumed of gas from the soil for seven days. Earthen pots were used for this experiment. Twenty four pots were filled with sterilized soil. Then the seedlings were transplanted in the pot. The pot soil was inoculated with *P. fluorescencs* suspension 5 days before transplanting of seedling and *R. solanacearum* (10^6 c.f.u./ml) was inoculated in that pot 5 days after transplanting. The experiment was conducted in completely randomized design (CRD) with two factors. The pots were arranged on the green house floor. Two variety of tomato viz. manik (V_1) and pusha rubi (V_2) were used. Following four treatments were used for this experiment:

T_0= Control (not treated with *P. fluorescens* suspension, treated with 10^6 c.f.u./ml *R. solanacearum* suspension)

T_1= Treated with *P. fluorescens* suspension which was 10 times diluted from stock suspension contained 10^9 c.f.u./ml

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T_2= Treated with *P. fluorescens* suspension which was 100 times diluted from stock suspension contained 10^8 c.f.u./ml

T_3= Treated with *P. fluorescens* suspension which was 1000 times diluted from stock suspension contained 10^7 c.f.u./ml

The treatment combinations were V_1T_0, V_1T_1, V_1T_2, V_1T_3, V_2T_0, V_2T_1, V_2T_2 and V_2T_3. Data were recorded after 30, 45 and 60 days of inoculation of *R. solanacearum,* on percentage of wilted plants and finally on yield. The collected data were statistically analyzed and mean values were adjusted by Duncan's new multiple range test (DMRT).

Results and Discussion

The varieties had no significant effect on bacterial wilt incidence in the green house condition. The highest wilt incidence was observed in pusa rubi (V_2) at 30, 45 and 60 days after inoculation (DAI) and the lowest wilt incidence was recorded in manik (V_1) at 30, 45 and 60 DAI. Significant difference on the yield of tomato was observed among the varieties. The highest yield (855.44 g) per plant was observed in V_1 and the lowest yield (794.65 g) was obtained from V_2 (Table 1).

Bacterial wilt incidence of tomato was significantly affected by the treatments at 30 DAI. The highest wilt incidence (55.00%) was recorded in T_0. The lowest wilt incidence (31.67%) was observed in T_1, this was followed by T_2 and T_3 having 35.00 and 40.00%. Statistically there was no significant difference between T_1 and T_2, also T_2 and T_3. But significant difference was present between T_1 and T_3 (Table 2). The significant effect of treatments on bacterial wilt incidence in green house condition was observed at 45 days after inoculation. T_0 resulted the highest wilt incidence (56.67%) and T_1 resulted the lowest wilt incidence (32.67%) which was followed by T_2 and T_3 having 36.33 and 41.50%, respectively. Statistically there was no significant difference between T_1 and T_2, also T_2 and T_3. But significant difference was present between T_1 and T_3. Bacterial wilt incidence was statistically different among the treatments at 60 DAI. The highest bacterial wilt incidence (57.17%) was observed in T_0. The lowest (33.83%) was observed in T_1 followed by T_2 and T_3 having 37.17 and 42.17%, respectively. Statistically there was no significant difference between T_1 and T_2, also T_2 and T_3. But significant difference was present between T_1 and T_3. Yield showed significant effect on the treatments. T_1 gave the highest yield (885.50 g) per plant, which was followed by T_2 and T_3 with 855.60 and 821.10 g, respectively. T_0 gave the lowest yield (738.00 g) per plant. All treatments were statistically different.

V_2T_0 showed maximum wilt incidence at 30, 45 and 60 DAI which was followed by V_1T_0. Minimum wilt incidence was observed in V_1T_1 at every stage which was followed by V_1T_2, V_2T_1 and V_2T_2. All the treatment combinations (V_1T_0, V_1T_1, V_1T_2, V_1T_3, V_2T_0, V_2T_1, V_2T_2 and V_2T_3) showed statistically significant response incase of yield of tomato. The highest yield (920.40 g) per plant was observed in V_1T_1 and the lowest yield (725.70 g) was observed in V_2T_0 (Table 3).

The varieties had no significant effect on bacterial wilt incidence of tomato. The results of this study revealed that *P. fluorescens* strain PF1 showed great impact on the bacterial wilt incidence. The *P. fluorescens* strain PF1 had tremendous effects on the reduction of bacterial wilt incidence of tomato in *R. solanacearum* infested pot soil. The maximum bacterial wilt incidence was recorded in T_0 (inoculated with only *R. solanacearum*) and the lowest bacterial wilt incidence was observed in T_1 at 30, 45 and 60 DAI. Bacterial wilt incidence increased with decreasing c.f.u/ml of *P. fluorescens.* For interaction effect between variety and treatment same tendency showed disease reduction, i.e. in every variety, disease decreased by increasing c.f.u/ml of *P. fluorescens*. The findings of this study are in agreement with the findings of Anuratha and Gnanamanikam. (1990) who reported that *P. fluorescens* protected tomato plants from wilt up to 95% in green house condition. Mulya *et al.* (1996) reported that *P. fluorescens* strain

Table 1: Effect of varieties on bacterial wilt and yield of tomato

Variety	Days after inoculation (%) 30	45	60	Yield/ plant (g)
V_1= Manik	39.17	40.50	41.75	855.44a
V_2= Pusa Ruby	41.67	46.08	50.42	794.65b
F-test	NS	NS	NS	*

Table 2: Effect of different treatments on bacterial wilt and yield of tomato

Treatments	Days after inoculation (%) 30	45	60	Yield/ plant (g)
T_0	55.00a	56.67a	57.17a	738.00d
T_1	31.67c	32.67c	33.83c	885.50a
T_2	35.00bc	36.33bc	37.17bc	855.60b
T_3	40.00b	41.50b	42.17b	821.10c
F-test	*	*	*	*

Table 3: Interaction effect of variety and treatment on bacterial wilt and yield of tomato

Treatment combinations	Days after inoculation (%) 30	45	60	Yield/ plant (g)
V_1T_0	53.33a	55.00a	55.67a	750.40f
V_1T_1	30.00c	31.00c	32.67c	920.40a
V_1T_2	33.33c	34.67c	36.33c	890.40b
V_1T_3	40.00b	41.33b	42.33b	860.60c
V_2T_0	56.67a	58.33a	58.66a	725.70g
V_2T_1	33.33c	34.33c	35.00c	850.50c
V_2T_2	36.67bc	38.00bc	38.00bc	820.70d
V_2T_3	40.00b	41.67b	42.00b	781.60e
F-test	*	*	*	*

Figures in a column with common letter(s) do not differ significantly at 1% level (*), V_1= Manik, T_0= Control (*R. solanacearum* 10^6 c.f.u./ml), V_2= Pusa Ruby f_3 T_1= *P. fluorescens* (10^9 c.f.u./ml), T_2 = *P. fluorescens* (10^8 c.f.u./ml), T_3= *P. fluorescens* (10^7 c.f.u./ml)

PfG32 isolated from the rhizosphere of onion actively suppressed the occurrence of bacterial wilt disease of tomato (caused by *R. solanacearum*) in vermiculite amended natural soil and produced antibiotic substance (s) and siderophores. Kumar *et al.* (2001) inoculated seed with five-plant growth promoting fluorescent Pseudomonas strains isolated from Indian and Swedish soils. In a synthetic culture medium, all the plant growth promoting fluorescent pseudomonads strains produced siderophores, which were shown to express antifungal and antibacterial activity. They suggested the potential use of these bacteria to induce plant growth and disease suppression in sustainable agriculture production systems.

The varieties had significant effect on yield of tomato. V_1 gave highest yield due to the variety had minimum wilt incidence. The *P. fluorescens* produced positive effect on yield. T_1 gave highest yield due to less bacterial wilt incidence plants and T_0 resulted lowest yield for presenting higher wilt incidence plants. Yield increased with increasing c.f.u/ml of *P. fluorescens.* For interaction effect, in every variety, same tendency was observed. The present findings are very relevant with the findings of Amara *et al.* (1996). They reported that tomato plant inoculated with plant growth promoting rhizobacteria (PGPR) *P. fluorescens* increased dry weight of shoot/plant, fruit weight and fruit yield. The findings are also in agreement with the findings of Gagne *et al.* (1993) who reported that *P. fluorescens* strain 63-28 significantly increased the marketable fruit yield by 13.3% and grade No.1 fruit weight/plant was increased by 18.2%. Kumar *et al.* (2001) inoculated seed with five-plant growth promoting fluorescent Pseudomonas strains isolated from Indian and Swedish soils. They suggested after study that the potential use of these bacteria to induce plant growth and disease suppression in sustainable agriculture production systems.

It is concluded that manik (V_1), T_1 (*P. fluorescens* 10^9 c.f.u./ml) and manik (V_1) x T_1 decreased bacterial wilt of tomato and increased fruit yield. So, V_1, T_1 and manik (V_1) x T_1 may be used in controlling bacterial wilt of tomato and increasing yield for tomato cultivation.

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