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Control of Bacterial Wilt of Tomato by *Pseudomonas fluorescens* in the Field

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Abstract: The experiments were conducted to evaluate the efficacy of antagonistic *P. fluorescens* in controlling wilt of tomato caused by *R. solanacearum* in the field. The tomato variety Manik showed 42.59% and Pusa Rubi showed 46.29% bacterial wilt. The lowest bacterial wilt incidence (35.18%) was recorded in T_2 (soil drenching of *P. fluorescens*) and highest incidence was in control plot. Plant height, number of branches/plant, number of fruits/plant, total fruit weight/plant and fruit yield (t ha^{-1}) was significantly highest in T_2 and lowest was in untreated control (T_0). Soil drenching by using *P. fluorescens* suspension contained 10^9 cfu/ml (T_2) may be used for controlling wilt and increasing yield of tomato.

Key word: Control, bacterial wilt, *Ralstonia solanacearum, Pseudomonas fluorescens*, 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 which is quite low compared to that of other tropical countries (FAO, 1999). There are so many reasons behind the low yield of tomato in our country. Among the reasons diseases caused by bacteria, fungi, virus and nematodes play a major role (Villarreal, 1980). Bacterial wilt of tomato caused by *Ralstonia solanacearum* is one of the potential threats to successful tomato cultivation. It is one of the major bacterial diseases of tomato affecting its growth and yield. It seriously affects the growth and yield of tomato. 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).

Ralstonia solanacearum causing bacterial wilt of tomato 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). *Pseudomonas 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). *Pseudomonus fluorescens* were found to be effective against *R. solanacearum* (Mulya *et al.*, 1996). Considering the above facts, the present study has been undertaken to evaluate the efficacy of antagonistic *P. fluorescens* in controlling wilt of tomato caused by *R. solanacearum* in the field.

Materials and Methods

The experiments were conducted at the field laboratory, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh during 2000 to 2001. Wilted tomato plants caused by *R. solanacearum* were collected from the farm of Bangladesh Agricultural University, Mymensingh. The presence of the 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 NA media by streaking. Three to four loop full of the virulent colonies were suspended in sterilized distilled water taken in screw cap tubes. The tubes were stored at ± 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.

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

Seeds of tomato cv. Manik were collected from Horticulture Research Centre, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur and another cv. Pusa Ruby 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. The collected seeds were sown on a 3x 1 m^2 seed bed.

The land was ploughed six times by power tiller. Total amount of cowdung (15 t/ha), triple super phosphate (175 kg/ha) and muriate of potash (150 kg/ha) were applied at the time of initial land preparation. Urea (250 kg ha^{-1}) was applied before 4 days of transplanting (Rashid, 1983). The seedlings were uprooted carefully with minimum damage. Healthy seedlings of equal height were selected for transplanting in the experimental plots.

The experiment was laid out following a split plot design with three replications. The tomato varieties Manik (V_1) and Push Ruby (V_2) were used as main plot and the following treatments were used as sub plot.

T_0 = Control (inoculated with *R. solanacearum* suspension which was 10 times diluted from stock suspension contained 10^6 c.f.u./ml to the base of the seedlings)

T_1= Seed treatment (Seeds treated with *P. fluorescens* suspension which was 10 times diluted from stock suspension contained 10^9 c.f.u./ml)

T_2= Soil drenching (by using *P. fluorescens* suspension which was 10 times diluted from stock suspension contained 10^9 c.f.u./ml)

T_3= Soil drenching by formulation (10 times diluted *P. fluorescens* stock suspension contained 10^9 c.f.u./ml + 250 g talc powder).

The suspension of *R. solanacearum* containing 10^8 c.f.u./ml were poured on to the base of the seedlings. The inoculation was done five days after using treatments. Data were recorded on bacterial wilt incidence, plant height, number of branches/plant, number of fruits/plant, total fruit weight/plant, fruit weight/plot and fruit yield (t ha^{-1}). The collected data was statistically analyzed and LSD test was done to evaluate the level of significance of the treatments (Zaman *et al.*, 1982).

Results and Discussion

The varieties had no significant effect on bacterial wilt incidence of tomato. Pusa Rubi showed 46.29% and Manik showed 42.59% bacterial wilt (Table 1). The lowest bacterial wilt incidence (35.18%) was recorded in T_2 followed by T_3 and T_1 having 38.89 and 42.59% incidence, respectively. The highest bacterial wilt incidence (61.11%) was observed in T_0 (control) (Table 1). Interaction effect of variety and treatment on bacterial wilt incidence in the field are presented in Table 1. 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 bacterial wilt incidence in the field. The lowest wilt incidence was obtained from V_1T_2 (33.33%) and the highest wilt incidence was obtained from V_2T_0 (62.96%). There were non significant differences between V_1T_1 and V_2T_3 both having 40.74% and V_1T_3 and V_2T_2 both having 37.03% bacterial wilt incidence.

Plant height between Manik (V_1) and Pusa Ruby (V_2) showed significant variation. Average plant height of V_1 and V_2 were found to be 43.03 and 39.26 cm, respectively. Number of branches/plant was found to be influenced by the variety. The number of branches/plant 8.34 was in V_1 and 9.60 in V_2. The two variety V_1 and V_2 did not differ significantly with each other in respect to number of fruits/plant, total fruit weight/plant and fruit yield (t/ha), rather they were identical in response (Table 2). Effect of different treatments on yield and yield attributes in the field are presented in (Table 2). Treatment effect was found to be highly significant in respect to plant height. Maximum plant height 56.86 cm was observed in T_2. Whereas minimum plant height 12.21 cm was recorded in T_0 (control). T_1 and T_3 gave plant height 44.59 and 50.92 cm, respectively. Maximum number of branches/plant (12.37) was observed in T_2 and minimum number of branches/plant (3.67) was observed in T_0 (control). Number of fruits/plant was highly influenced by different treatments. Treatment T_2 was found to be the best among four treatments which gave 12.80 number of fruits/plant and control treatment gave the lowest (3.88) number of fruits/plant. Maximum total fruit weight per plant 1297.52 g was found in T_2 and lowest (266.30) was found in T_0 (control). Fruit yield was significantly governed by different treatments. Maximum yield 51.89 tons/ha was recorded in T_2 and minimum 10.63 tons/ha was obtained from T_0 (control). Interaction effect of varieties and treatments on yield and yield attributes in the field are presented in (Table 3). 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) had significant effect on number of branches/plant, number of fruits/plant, total fruit weight/plant and fruit yield in the field. The highest plant height (60.88 cm) was obtained in V_1T_2 and the lowest was observed in V_2T_0 with 10.69 cm. Maximum number of branches per plant (13.00) was counted in V_2T_2 and minimum (3.17) was counted in V_1T_0. There were non significant difference between V_2T_1 and V_2T_3 having 10.57 and 10.67, respectively. The highest fruits per plant (12.93) were obtained from V_1T_2 which was statistically identical with V_2T_2 having 12.67. The lowest fruits per plant (4.50) was obtained from V_1T_0. The highest fruit weight per plant was obtained in V_1T_2 having 1310.64 g and the lowest was V_2T_0 having 223.02 g. The highest yield (52.39 t ha^{-1}) was obtained from V_1T_2 which followed by V_2T_2 having 51.89 t ha^{-1} and the lowest yield (12.37 t ha^{-1}) was obtained from V_1T_0.

The varieties had non significant effect on bacterial wilt incidence of tomato. The results of the present study revealed that *Pseudomonas fluorescens* strain PF1 showed great impact on the prevention of bacterial wilt incidence. The *Pseudomonas*

Table 1: Effect of varieties different treatments and their interaction on bacterial wilt incidence in the field

Varieties	% Bacterial wilt
V_1= Manik	42.59
V_2= Pusa Rubi	46.29
LSD (P= 0.05)	NS
Treatments	
T_0	61.11a
T_1	42.59b
T_2	35.18b
T_3	38.89b
LSD (P= 0.01)	9.2
V_1T_0	59.25b
V_1T_1	40.74d
V_1T_2	33.33f
V_1T_3	37.03e
V_2T_0	62.96a
V_2T_1	44.44c
V_2T_2	37.03e
V_2T_3	40.74d
LSD (P= 0.05)	1.439

Figures in a column with common letters do not differ significantly at 5% level of probability

Table 2: Effect of varieties and different treatments on yield and yield attributes in the field

Variety	Plant height (cm)	No. of branches/plant	No. of fruits/plant	Total fruit wt/plant (g)	Fruit yield (t ha^{-1})
V_1= Manik	43.03a	8.34b	9.95	889.35	35.55
V_2= Pusa Rubi	39.26b	9.60a	9.28	836.38	33.46
LSD (P= 0.01)	1.13	0.72	NS	NS	NS
Treatments					
T_0	12.21d	3.67c	3.88c	266.30c	10.63c
T_1	44.59c	9.69b	11.11b	910.25b	36.40b
T_2	56.86a	12.37a	12.80a	1297.52a	51.89a
T_3	50.92b	10.17b	10.67b	977.38b	39.08b
LSD (P= 0.01)	2.75	1.16	1.25	106.90	4.28

Figures in a column with common letters do not differ significantly at 5% level of probability NS= Non significant

Table 3: Effect of interaction (variety x treatment) on yield and yield attributes in the field

Treatment combinations	Plant height (cm)	No. of branches/plant	No. of fruits/plant	Total fruit wt/plant (g)	Fruit yield (t ha^{-1})
V_1T_0	13.72e	3.17g	4.50e	309.58d	12.37e
V_1T_1	46.05c	8.81e	11.42b	932.02c	37.27c
V_1T_2	60.88a	11.73b	12.93a	1310.64a	52.39a
V_1T_3	51.48b	9.67d	10.93c	1005.15b	40.17b
V_2T_0	10.69f	4.17f	3.27f	223.02e	8.90f
V_2T_1	43.12d	10.57c	10.80cd	888.47c	35.53d
V_2T_2	52.84b	13.00a	12.67a	1284.40a	51.39a
V_2T_3	50.35b	10.67c	10.40d	949.62bc	38.00c
LSD (P= 0.01)	2.81	0.1751	0.4144	61.91	1.082

Figures in a column with common letters do not differ significantly at 1% level of probability *fluorescens* strain PF1 had tremendous effects on the reduction of bacterial wilt incidence of tomato in *R. solanacearum* infested 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_2 (treated with both *R. solanacearum* and *P. fluorescens*). Bacterial wilt was also lower in T_1 (seed treated with *P. fluorescens*) and in T_3 (soil treated with talc + *P. fluorescens*). The findings of the present study are in agreement with those of Anuratha and Gnanamanikam (1990) who reported that *P. fluorescens* effectively controlled bacterial wilt of tomato in the field. Mulya *et al.* (1996) reported that *P. fluorescens* strain 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. The suppression of bacterial wilt of tomato by PfG32 was correlated to the suppression of the pathogen population on root surfaces and to the delay is appearance of a detectable population of the pathogen in root tissue. 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 *Pseudomonas* 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 *Pseudomonas fluorescens* produced positive effect on the plant growth characters such as plant height, number of branches/plants. The maximum plant height was recorded in plants grown in treatment T_2 (10 times dilution of *P. fluorescens* stock suspension) and minimum plant height was recorded in plants grown in treatment T_0 (inoculated with only *R. solanacearum*). Highest number of branches/plant was observed in plants grown in plots treated with T_2 (10 times dilution of *P. fluorescens* stock suspension) and lowest in T_0 (inoculated with only *R. solanacearum*). Anuratha and Gnanamanikam (1990) reported that plants treated with *P. fluorescens* increased the plant height and biomass values compared with untreated plants. Amara *et al.* (1996) also reported that number of branches/plant was increased by treating with *P. fluorescens*. The tested *P. fluorescens* strain PF1 showed greater impact on number of fruits/plant, total fruit weight/plant and fruit yield (t ha^{-1}). In respect of all these parameters maximum value was recorded in T_2 and minimum value was recorded in treatment T_0. 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 has been clearly observed that T_2 (Soil drenching with *P. fluorescens* suspension contained 10^9 c.f.u./ml) and/or treatment combination V_1 (Manik) x T_2 decreased wilt of tomato and increased plant height, number of branches/plant, number of fruits/plant, total fruit weight/plant and fruit yield (t ha^{-1}). So, V_1, T_2 and V_1T_2 may be used in controlling wilt of tomato and increasing yield and yield attributes for tomato cultivation.

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