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RK Bannihatti
Department of Plant Pathology, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra, India

AP Suryawanshi
Department of Plant Pathology, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra, India

Correspondence
RK Bannihatti
Department of Plant Pathology, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra, India

Integrated management of bacterial wilt of tomato caused by *Ralstonia solanacearum*

RK Bannihatti and AP Suryawanshi

Abstract

Bacterial wilt caused by *Ralstonia solanacearum* (Smith) Yabuuchi is one of the most destructive diseases of tomato (*Lycopersicum esculentum*), causing accountable losses of about 10-90 per cent. Present investigations on the disease (*R. solanacearum*) were carried out during 2014-15 to fulfill the objectives defined, at the Department of Plant Pathology, College of Agriculture, VNMKV, Parbhani. A total of 12 treatments comprising most effective (based on plate/pot culture studies) antibiotics, fungicides, bioagents, organic amendments and phytoextracts were attempted (alone or in combination) for the integrated management of bacterial wilt pathogen (*R. solanacearum)*, by applying sick soil technique. Among the treatments, Streptocycline + *P. fluorescens* + vermicompost were found most effective with significantly highest germination (78.32%), respectively, followed by Streptocycline + Copper oxychloride (73.29 %) and *T. horizanum+ P. fluorescens* (68.30 %). All the test amendments recorded significant reduction in least average mortality (PEM and Wilt) over untreated control. However highest least average mortality reduction was recorded with Streptocycline + *P. fluorescens* + vermicompost were found most effective with significantly least reduction (65.73 and 60.27 %), respectively, followed by Streptocycline + Copper oxychloride (59.88 and 54.23 %) and *T. horizanum+ P. fluorescens* (52.38 and 47.32 %) respectively. The result of the studies revealed that all the treatments attempted showed significantly highest seed germination, lowest average incidence (PEM and wilt) and highest reduction of average incidence (PEM and wilt), over untreated control.

Keywords: tomato, bacterial wilt, antibiotics, biocontrol agents, phytoextracts, organic amendmens

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most widely grown fruit vegetable in the world, with third rank in priority after Potato and Onion in India but ranks second after potato in the world. India ranks second in the area as well as in production of Tomato. Commercially grown throughout the world for fresh fruit, market and processing industries. China is the largest tomato producing country in the world, followed by India and USA (Anonymous, 2014) [1]. In India, the area under tomato cultivation was 880 thousand hectare with production of 18227 thousand MT and productivity of 20.7MT/ha (Anonymous, 2013-14) [1]. The Maharashtra state is the fourth largest tomato producer in the India with an area of 50 thousand hectare, production of 1050 thousand MT and productivity 21MT/ha (Anonymous, 2013-14) [1]. Other leading tomato producing states are: Andra Pradesh, Karnataka and Orrisa.

In the tropics, tomato production is severely constrained by disease and insect pests. Tomato crop is being affected by many fungal, bacterial, viral and nematode diseases such as bacterial wilt [*Ralstonia solanacearum* (Smith) Yabuuchi], bacterial leaf spot (*Xanthomonas campestris pv. vesitocoria*), bacterial canker (*Clavibacter michiganensis pv. michiganensi*), early blight (*Alternaria solani*), powdery mildew (*Leveillula taurica*) Tomato mosaic virus, Tomato leaf curl virus and Tomato spotted wilt (viruses) and root knot nematode (*Meloidogyne incognata*). Among these diseases, bacterial wilt caused by *Ralstonia solanacearum* (Smith.) Yabuuchi (formerly *Pseudomonas solanacearum*) is one of the most economically important and devastating disease of tomato crop. The disease was first reported from Asia and South America. This disease is of common occurrence whenever solanaceous crops *viz* tomato, brinjal, potato and chilli etc are grown and is more severe under weather conditions of high temperature and high humidity, congenial for disease development (Sunder *et al*., 2011) [23]. In India bacterial wilt of tomato was first reported in Solan area of Himachal Pradesh (Gupta *et.al*., 1998) [5] *R. solanacearum* (Smith) is a serious soil borne pathogen of solanaceous vegetable crops grown during summer, rainy and winter seasons.

Tomato (*Lycopersicon esculentum*) is one of the important solanaceous vegetables, which suffers badly due to *R solanacearum*, wherever high temperature (28 to 36ºC) and high moisture (50 to 100 %) prevails (Sharma *et al.* 2009) [20]. In India about 10 to 100% incidence of tomato bacterial wilt during the summer were reported (Kishun, 1985) [8]. *R. solanacearum* is a globaly dispersed and heterogeneous bacterial pathogen, with socioeconomic impacts (Yabuuchi *et al*. 1995) [26].

Material and Methods

Those antibiotics, antibacterial fungicides, bioagents, botanicals and amendments found most effective against *R solanacearum* during present *in vitro* studies (plate and pot culture) were selected and assessed for integrated management of bacterial wilt (*R solanacearum*) of tomato (pot culture). The earthen pots (30 cm dia.) disinfected with 5 per cent solution of Copper sulphate were filled with autoclaved potting mixture of soil: sand: FYM (2:1:1). The mass multiplied Nutrient broth pure culture (48 hrs old) of the test bacterium (2×10^8cfu/ml) was drenched (@ 50ml/kg potting mixture) by spreading uniformly on potting mixture in the pots, watered adequately and incubated for 96 hrs in screen house to proliferate the test bacterium and make the soil/potting mixture sick.

A total of 11 treatments were attempted as seed treatment (ST) with test antibiotics, soil drenching (SD) of test bioagents and phytoextracts after 72 hrs of sowing and soil application (SA) before 48 hrs of sowing. The seeds of susceptible tomato Cv. Pusa Ruby treated with the test antibiotics (T_1, T2, T_3, T9 and T_{11}) and untreated healthy seeds (T_4, T_5, T_6, T_7, T_8, and T_{10}) were sown (20 seeds/pot) in pots containing *R. solanacearum* sick soil.

Surface sterilized (0.1 % $HgCl_2$) healthy seeds of tomato cv. Pusa Ruby were sown (20 seeds / pot) in the earthen pots containing *R solanacearum* sick soil / potting mixture and maintained as untreated control. All these pots (treated and untreated) were watered regularly and maintained in the screen house for further observations.

Results and Discussion

A total of 12 treatments comprising most effective (based on plate/pot culture studies) antibiotics, fungicides, bioagents, organic amendments and phytoextracts were attempted (alone or in combination) for the integrated management of bacterial wilt pathogen (*R solanacearum)*, by applying sick soil technique. The results recorded on seed germination, pre-emergence mortality (PEM) and wilting are presented (Tables 2) described and discussed here in following paragraphs.

Seed germination

Results (Table 2 and Fig) revealed that all the treatments exhibited improved seed germination, over untreated control and it was ranged from 45.00 to 78.32 per cent, as against 33.33 in untreated control. However, significantly highest seed germination was recorded in the treatment (Streptocycline + *P. fluorescens* + vermicompost) with 78.32 per cent, followed by Streptocycline + Copper oxychloride (73.29%), *T. horizanum* + *P. fluorescens* (68.30%). streptocycline (63.30 %), *P. fluorescens* (60.65%) Copper oxy chloride (60.00%), *T. horizanum* (56.67%), vermicompost (51.67%), gentamycin (50.00%) and acetone garlic clove extract (48.00 %). Whereas, significantly least seed germination was recorded with aqueous garlic clove extract (45.00%) but more than that of untreated control (33.33 %).

Pre emergence mortality

Results (Table 2 and Fig) revealed that all test amendments significantly influenced the pre-emergence mortality (PEM), against *R solanacearum* and it was ranged from 21.59 to 55.00 per cent, as against 66.67 per cent in untreated control. However, significantly lowest pre emergence mortality (PEM) recorded in the treatment Streptocycline+ *P. fluorescens* + vermicompost (21.59%), followed by Streptocycline + Copper oxychloride (26.65%), *T. harizanum+ P. fluorescens* (31.70%), *P. fluorescens* (33.35%), Streptocyclin (36.57%), Copper oxychloride (40.00%), *T. horizanum* (43.40 %), and Gentamycin (50.00%). Rest of the treatments acetone garlic clove extract and aqueous garlic clove extracts were found comparatively less effective with maximum PEM with the 51.68 to 55.00 per cent, respectively.

Percent wilt

The percentage wilting recorded in all the treatments was ranged from 27.78 to 59.25 per cent, as against 69.83per cent in untreated control. However, significantly lowest per cent wilt recorded with treatments Streptocycline+ *P. fluorescens* + vermicompost (27.78 %) and Streptocycline + Copper oxychloride (31.74 %) respectively, both which were at par ; followed by *T. harizanum+ P. fluorescens* (36.63 %), *P. fluorescens* (37.54 %), streptocycline (42.09%), copper oxychloride (44.45 %), *T. harizanum* (49.74), vermicompost (51.52 %) and gentamycin (55.18 %). Rest of the treatments acetone garlic clove extract and aqueous garlic clove extracts were found comparatively less effective with highest percent wilting 57.40 to 59.25 respectively.

Average incidence (PEM and wilt) in all treatments recorded was ranged from 24.68 to 57.12 per cent, as against 68.25 per cent untreated control. However, significantly lowest incidence with the treatment streptocycline + *P. fluorescens* + vermicompost (24.68%) and streptocycline + copper oxychloride (29.19%) respectively, both which were at par ; followed by *T. harizanum+ P. fluorescens* (34.16 %), *P. fluorescens* (35.44 %), streptocycline (39.38%), copper oxychloride (42.22 %), *T. harizanum* (45.19), vermicompost (49.96 %) and gentamycin (52.42 %). Rest of treatments acetone and aqueous garlic clove extracts were recorded highest average incidence 54.55 and 57.12 percent, respectively.

Reduction in pre emergence mortality and wilt

Results (Table 2) indicated that all treatments attempted for integrated managements were found effective in reducing both pre-emergence mortality and percent wilting over untreated control.

Reduction in pre emergence mortality

The per cent reduction in PEM recorded in treatments was ranged from 17.44 to 65.73per cent. However, significantly reduction in PEM with treatment Streptocycline + *P. fluorescens* + vermicompost (65.73%) and Streptocycline + Copper oxychloride (59.88%), both which were at par; followed by *T. harizanum* + *P. fluorescens* (52.38 %), *P. fluorescens* (50.00 %), Streptocycline (44.87%), Copper oxychloride (39.32%), *T. harizanum* (34.53%), vermicompost (27.47 %) and Gentamicin (26.55%). Rest of the treatments acetone and aqueous garlic clove extracts were found least effective with lowest reduction in PEM 22.34 and 17.44 percent, respectively.

Reduction in wilt incidence

The reductions in per cent wilt recorded in treatments were ranged from 14.96 to 60.27 per cent, over untreated control. However, significantly highest reduction per cent wilt with treatments Streptocycline+ *P. fluorescens* + vermicompost (60.27 %) and Streptocycline + Copper oxychloride (54.23 %) respectively, both which were at par ; *T. harizanum* + *P. fluorescens* (47.32%), *P. fluorescens* (45.90%), Streptocycline (39.52%), Copper oxychloride (36.38%), *T. harizanum* (29.19%), vermicompost (26.20%) and Gentamycin (21.53%). Rest of the treatments acetone and aqueous garlic clove extracts were found least effective with minimum reduction in per cent wilting 14.96 to 17.40 per cent, respectively.

Reduction in the average incidence (PEM and wilt) recorded in treatments was ranged from 16.20 to 63.00per cent. However, significantly highest reduction in average incidence with treatment Streptocycline + *P. fluorescens* + vermicompost (63.00%) and Streptocycline + Copper oxychloride (57.15%) both which were at par ; followed b *T. harizanum*+ *P. fluorescens* (49.99%), *P. fluorescens* (47.95%), Streptocycline (42.19%), Copper oxychloride (38.15%), *T. harizanum* (32.36%), vermicompost (26.83%) and Gentamycin (24.04%). Rest of the treatments acetone and aqueous garlic extracts found least effective with least reduction in average incidence 16.20 to 19.87 per cent, respectively.

Results (Table 2) obtained in the present study on integrated management of bacterial wilt (*R. solanacearum*) of tomato indicated that all the treatments attempted significantly enhanced the seed germination, reduced the pre emergence mortality and wilt incidence, over untreated control. These results of the present study obtained on the integrated bioefficacy of various treatments (antibacterial chemicals, botanicals, bioagents and amendments) against bacterial wilt of tomato and their effects on improved seed germination and reduced pre emergence mortality as well as wilt incidence are in conformity with those reported earlier by several workers (Kumar, 1970; Shekawat *et al*., 1987; Karuna and Khan, 1993; Mazumadar, 1998; Sharma and Kumar, 2000; Kumar and Sood, 2001; Kumar and Sood, 2003; Dubey, 2005; Venkatesh, 2005; Biswas and Singh 2008; Ramesh, 2008; Sharma and Kumar, 2009a; Sharma and Kumar, 2009b; Vanitha *et al*., 2009) [12, 21, 7, 13, 10, 11, 4, 25, 15, 19, 18, 20, 24] Murthy and Srinivas, 2012 [14]; Reddy *et al*., 2012 [16] and Sawant *et al*., 2014) [17].

Table 1: Treatment details of experiment

T. No	Treatments	Concentrations (ppm or ml or kg)
T_1	Gentamicin (500ppm)	ST @ 20ml/kg seed
T_2	Streptocycline (500ppm)	ST @ 20ml/kg seed
T_3	Copper oxychloride (50 WP)	ST @ 3g/kg seed
T_4	*T. horizanum* (2×10^7 cfu/ml)	SD @ 25 ml/kg soil
T_5	*P. fluorescens* (2×10^8 cfu/ml)	SD @ 25 ml/kg soil
T_6	Aqueous garlic bulb extract (20%)	SD @ 25 ml/kg soil
T_7	Acetone garlic bulbs extract (20%)	SD @ 25 ml/kg soil
T_8	Vermicompost	SA @ 50g/ kg soil
T_9	Streptocycline (500ppm) + Copper oxychloride	ST @ 20ml + 3g/kg seed
T_{10}	*T. horizanum* + *P fluorescens*	SD @ 20 ml / kg soil
T_{11}	Streptocycline (500ppm) + *P fluorescens*+ Vermicompost	ST @ 20ml/kg seed +SD @ 25 ml /kg soil + SA 50 g/ kg soil
T_{12}	Control (untreated)	---

Table 2: Efficacy of various treatments integrated for management of tomato bacterial wilt (*R solanacearum*)

T. No.	Treatments	Rate	Germination * (%)	Incidence *(%)		Av. (%)	Reduction over control (%)		Av. (%)
				PEM	Wilt		PEM	Wilt	
T_1	Gentamycine	ST @ 20ml/kg seed	50.00 (45.00)	50.00 (45.00)	55.18 (47.97)	52.42 (46.39)	26.55 (31.02)	21.53 (27.65)	24.04 (29.36)
T_2	Streptocycline	ST @ 20ml/kg seed	63.30 (52.71)	36.67 (37.27)	42.09 (40.45)	39.38 (38.87)	44.87 (42.06)	39.52 (38.95)	42.19 (40.51)
T_3	Copper oxychloride	ST @ 3g/kg seed	60.00 (50.77)	40.00 (39.23)	44.45 (41.81)	42.22 (40.52)	39.32 (38.83)	36.38 (39.10)	38.15 (38.15)
T_4	*T. horizanum*	SD @ 25 ml/kg soil	56.67 (48.83)	43.40 (41.21)	49.74 (44.85)	45.19 (42.24)	34.53 (35.99)	29.19 (32.70)	32.36 (34.67)
T_5	*P. fluorescens*	SD @ 25 ml/kg soil	60.65 (51.15)	33.35 (35.27)	37.54 (37.78)	35.44 (36.54)	50.00 (45.00)	45.90 (42.65)	47.95 (43.83)
T_6	Aqueous garlic bulb extract (20%)	SD @ 25 ml/kg soil	45.00 (42.13)	55.00 (47.87)	59.25 (50.33)	57.12 (49.09)	17.44 (24.68)	14.96 (22.75)	16.20 (23.73)
T_7	Acetone garlic bulb extract (20%)	SD @ 25 ml/kg soil	48.00 (43.85)	51.68 (45.96)	57.40 (49.26)	54.55 (47.71)	22.34 (28.21)	17.40 (24.65)	19.87 (26.47)
T_8	Vermicompost	SA 50g/ kg soil	51.67 (45.96)	48.40 (44.08)	51.52 (45.87)	49.96 (44.98)	27.47 (31.61)	26.20 (30.79)	26.83 (31.20)
T_9	Streptocycline + copper oxychloride	ST @ 20ml + 3g/kg seed	73.29 (58.88)	26.65 (31.08)	31.74 (34.29)	29.19 (32.70)	59.88 (50.70)	54.43 (47.54)	57.15 (49.11)
T_{10}	*T. horizanum* + *P fluorescens*	SD @ 20 ml / kg soil	68.30 (55.73)	31.70 (34.27)	36.63 (37.25)	34.16 (53.57)	52.38 (46.36)	47.42 (43.52)	49.99 (44.99)
T_{11}	Streptocycline + *P fluorescens* + Vermicompost	ST @ 20ml/kg seed +SD @ 25 ml /kg soil + SA 50 g/ kg soil	78.32 (62.25)	21.59 (27.69)	27.78 (31.81)	24.68 (29.79)	65.73 (54.17)	60.27 (50.93)	63.00 (62.54)
T_{12}	Control (Untreated)		33.33 (35.26)	66.67 (54.74)	69.83 (56.68)	68.25 (59.70)	--	--	--

		SE ±	1.62	1.62	2.85	-	3.09	2.79	-
		CD (P=0.01 %)	4.86	4.86	8.32	-	9.01	8.15	-

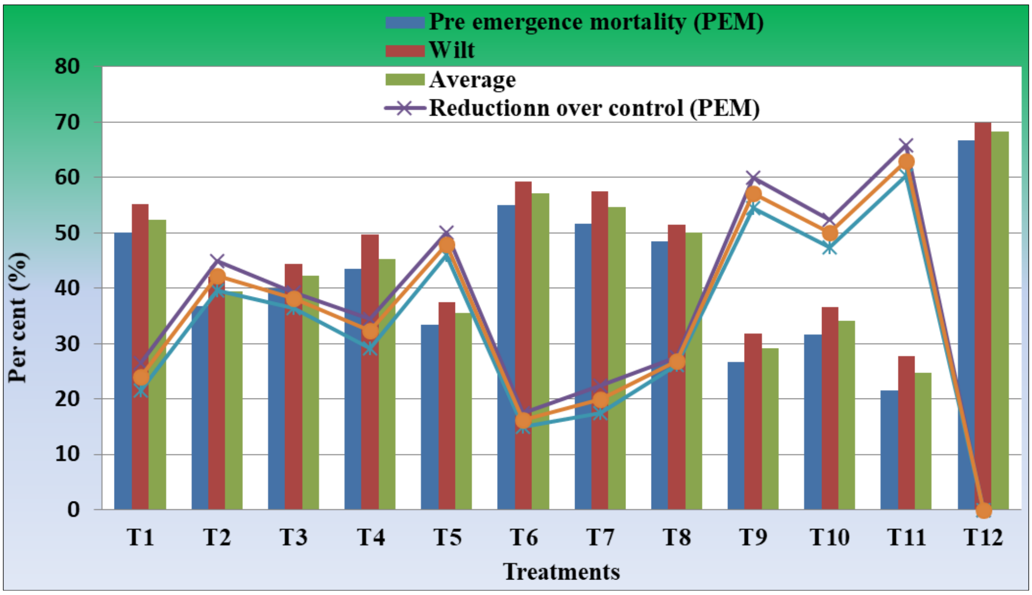

Fig 1: Efficacy of various treatments integrated for management of tomato bacterial wilt (*R solanacearum*)

T_1 Gentamicin	T_7 Acetone garlic bulb extract (25%)
T_2 Streptocycline	T_8 Vermicompost
T_3 Copper Oxychloride	T_9 Streptocycline + copper oxychloride
T_4 *T. horizanum*	T_{10} *T. horizanum* + *P fluorescens*
T_5 *P. fluorescens*	T_{11} Streptocycline+ *P fluorescens*+ Vermicompost
T_6 Aqueous garlic bulb extracts (25%)	T_{12} Control

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