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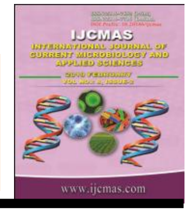

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Evaluation of Different Soil Amendments and Germplasm / Varieties against Tomato Bacterial Wilt Caused by *Ralstonia solanacearum*

R.K. Bannihatti*, A.P. Suryawanshi, K.S. Sayyed and V.B. Bhujabal

Department of Plant Pathology, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani-431402, Maharashtra, India

**Corresponding author*

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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 10 organic amendments evaluated (pot culture) as pre- sowing soil applications were found effective against *R. solanacearum.* However, significantly highest seed germination was recorded with vermicompost (76.50 %), followed by karanj cake (71.74 %), neem seed cake (66.75 %) and compost (61.67%); whereas, significantly highest reduction in average incidence (PEM and wilt) was recorded with vermicompost (60.66%), followed by karanj cake (53.74%), neem seed cake (46.70 %) and compost (41.33%). Under artificial epiphytotic conditions (root zone drenching method), all the 14 tomato entries evaluated exhibited different reactions against *R. solanacearum*. However, six entries (Tom-21, Tom-4, Tom-17, Tom-13, S-22 and PKM-1) were moderately resistant with average wilt incidence in the range of 25.28 to 33.25 per cent; four entries (Tom-15, Tom-11, Tom-27 and Tom-7) were moderately susceptible with average wilt incidence in the range of 47.81 to 52.46 per cent and four entries (Tom-8, Tom-18, Tom-2 and Pusa Ruby) were susceptible with average wilt incidence in the range of 61.13–78.50 per cent.

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). 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). 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). 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 compestris pv. vesitocoria*), bacterial canker (*Clavibacterm ichiganensi spv. michiganensi*), early blight (*Alterneria 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 (Smith, 1880). This disease is of common occurrence whenever solanaceous crops *viz* tomato, brinjal, potato and chillietc are grown and is more severe under weather conditions of high temperature and high humidity, congenial for disease development (Sunder *et al.*, 2011).

In India bacterial wilt of tomato was first reported in Solan area of Himachal Pradesh(Gupta *et al*.,1998*) 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). In India about 10 to 100% incidence of tomato bacterial wilt during the summer were reported (Kishun, 1985). *R. solanacearum* is a globaly dispersed and heterogeneous bacterial pathogen, with socioeconomic impacts (Yabuuchi *et al.*, 1995).

Materials and Methods

Bioefficacy of organic amendments

A total of 9 organic amendments were evaluated against *R solanacearum* by sick soil method in pot culture, under screen house conditions. The test amendments were applied as presowing treatment (protective). Except vermicompost, all the test amendments were crushed physically to coarse form and used for soil application. 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 (48 hr old nutrient broth culture: 2 x10^8 cfu/ml) of *R solanacearum* was drenched (@ 50 ml/ kg potting mixture) evenly to the potting mixture in pots, these pots were incubated for 96 hrs in screen house to proliferate the bacterium and make the soil / potting mixture sick. The coarse ground test amendments were applied (@ 50 g / kg mixture) in the earthen pots containing test bacterium sick soil/ potting mixture, mixed thoroughly, watered regularly and maintained in screen house. After 72 hrs of amendments application, surface sterilized (0.1 % $HgCl_2$) healthy seed of tomato Cv. Pusa Ruby were sown (20 seeds/pot), watered regularly and maintained in the screen house. Three pots / treatment / replication were maintained. The earthen pots containing *R solanacearum* sick soil and sown with surface sterilized healthy seed of tomato cv. Pusa Ruby, without amendment were maintained as untreated control.

Experimental details

Design	: CRD
Replications	: Three
Treatments	: Ten
Variety	: Pusa Ruby

Treatment details

T_1: Compost
T_6: Groundnut cake
T_2:Poultry manure T_7: Sunflower cake
T_3:Vermicompost T_8: Cotton seed cake
T_4:Goatmanure T_9: Neem seed cake
T_5:Karanja cake T_{10}: Control (untreated)

Observations on seed germination and pre-emergence mortality (PESR) were recorded at seven days after sowing and that of wilting were recorded at 30th and 45 DAS. The per cent seed germination, pre-emergence mortality (PEM) and wilting were calculated by following formulae:

Germination (%) =

$$\frac{\text{No. of seeds germinated}}{\text{Total no. of seeds sown}} \times 100$$

$$\text{PEM (\%)} = \frac{\text{No. of seeds ungerminated/rotted}}{\text{Total no. of seeds sown}} \times 100$$

$$\text{Wilting (\%)} = \frac{\text{No. of seedlings died/wilted}}{\text{Total no. of seedlings}} \times 100$$

$$\text{Reduction (\%) in PEM / Wilting} = \frac{C-T}{C} \times 100$$

Where,
C= Per cent mortality /wilting in treatment pots
T = Per cent mortality / wilting control pots

Screening of tomato entries

Fifteen days old seedlings of the 14 test entries were transplanted (5 seedlings/pot/entry) in the earthen pots (30 cm dia.) filled with steam sterilized potting mixture of soil: sand: FYM (2:1:1), maintained in screen house and watered regularly.

Two pots/ entry/replication were maintained. One week after transplanting these potted seedlings were inoculated by drenching at root zone with 48hr old pure culture suspensions of *R.soalancearum* (2×10^8cfu/ml) and maintained by watering frequently, under screen house.

Observations on bacterial wilt incidence were recorded applying 0-5 grade disease rating scale (Winsted and Kelman, 1952) at 30 and 45 days after inoculation of pathogen. The data was averaged and percent bacterial wilt disease incidence was calculated by following formula.

Percent disease incidence (PDI) =

$$\frac{\text{Number of plants showing wilts symptoms}}{\text{Total number of plants}} \times 100$$

Disease rating scale

Grade	% Incidence	Disease Reactions
0	Highly resistant (HR)	Plants did not show any wilt symptom
1	Resistant (R)	1 - 20 % plants wilted
2	Moderately resistant (MR)	21- 40 % plants wilted
3	Moderately susceptible (MS)	41- 60 % plants wilted
4	Susceptible (S)	61- 80% plants wilted
5	Highly susceptible (HS)	More than 80% plant wilted

Experimental details

Design : C.R.D.
Replications : Three
Treatments : Fourteen

Tr. No	Treatments	Tr. No	Treatments
T_1	Tom-5	T_8	Tom 18
T_2	Tom 11	T_9	Tom 2
T_3	Tom 21	T_{10}	Tom 17
T_4	Tom 8	T_{11}	Tom 13
T_5	Tom 27	T_{12}	S-22
T_6	Tom 7	T_{13}	PKM-1
T_7	Tom 4	T_{14}	Pusa Ruby

Statistical analysis

The data obtained in all the experiments was statistical analyzed. The percentage values were transformed into arcsine values.

The standard error (S.E.) and critical difference (C.D.) at level P = 0.01 were worked out and results obtained were compared statistically. All the statistical analysis was done using VNMKV-STAT statistical programmer at Central Computer Laboratory, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani.

Results and Discussion

In *vitro* bioefficacy of organic amendments

A total of 9 amendments were evaluated as pre- sowing soil application to assess their efficacy against *R solanacearum*, employing sick soil technique and sowing susceptible tomato cv. Pusa Ruby in pot culture under glass house conditions and the results obtained on seed germination, pre-emergence mortality and wilting are presented in the Table 1 and Fig.1.

Seed germination

Results (Table 1) revealed that all the test amendmentssignificantly improved the per cent seed germination, over untreated control and it was ranged from 40 to 76.50 per cent, as against 35 per cent in untreatedcontrol. However, vermicompost and karanj cake were found most effective with significantly highest seed germination of 76.50 and 71.74 per cent, respectively and both were at par. These were followed by the amendments *viz*.,neem seed cake (66.75 %),compost (61.67 %),sunflower cake (56.72%), Goat manure (51.55) and Cotton seed cake (48.25%) respectively and later two were at par. The amendments *viz*., poultry manureand groundnut cakes were found comparatively least effective with minimum germination of 40.0 and 43.29 per cent, respectively.

Pre emergence mortality

Results (Table 1 and Fig) revealed that all the test amendmentssignificantly influenced the pre-emergence mortality (PEM) and it was ranged from 23.33 to 60.00 per cent, as against 65.00 per cent in untreated control. However, vermicompostwas found most effective with significantly least pre-emergence mortality (23.33 %), followed bykaranj cake (28.40%), neem seed cake (33.25%), compost (38.33%),sunflower cake (43.41%), goat manure (48.29%)and Cotton seed cake (51.67%). Rest of the amendments recorded pre emergence mortality 56.67 to 60.00 per cent respectively as against 65.00 per cent in control.

Wilt incidence

Percent wilting recorded with all the test amendments was from 30.55 to 65.27 per cent, as against 71.42 per cent in untreated control. However, vermicompost was found most effective with significantly least wilting per cent (30.55 %), followed by amendments karanj cake (34.92) and neem seed cake(36.65 %) both were at par; compost (40.59%), sunflower cake (50.25%), goat manure (51.51%) and cotton seed cake (55.55%). Rest of the amendments groundnut cake and poultry manure were least effective and recorded comparatively maximum wilting per cent in the range of 61.57 to 65.27 per cent, respectively.

Average (PEM and Wilt) recorded in all the test amendments were ranged from 26.94 to 62.63 per cent, as against 68.21 per cent in untreated control.

However, comparatively minimum average incidence was recorded with vermicompost (26.94%), followed by karanj cake (31.66%), neem seed cake (35.00%), compost (39.46%), sunflower cake (46.83%), goat manure (49.90%) and cotton seed cake (53.61 %). Rest of the amendments groundnut cake and poultry manure were recorded average incidence in the range of 59.12 to 62.63 per cent, respectively.

Reduction in mortality and wilt incidence

All test amendments recorded significant reduction in pre -emergence mortality (PEM) over untreated control. Reduction in pre emergence mortality recorded was ranged from 7.67 to 64.10 per cent.

However, significantly highest reduction in pre emergence mortality (PEM) was recorded in vermicompost (64.10%), followed karanj cake (56.40%), neem seed cake (48.72%), compost (39.42%), sunflower cake (33.33%), goat manure (25.63%) and cotton seed cake (21.81 %). Rests of the amendments groundnut cake and poultry manure were recorded least reduction pre emergence mortality in the range of 12.82 to 7.90 per cent. All test amendments recorded significant reduction in percent wilting over control. Reduction in percent wilting recorded was ranged from 8.65 to 57.22 per cent. However, significantly highest per cent reduction in wilting recorded in vermicompost (57.22 %), followed by karanj cake (53.73%), neem seed cake (45.20 %), compost (43.15%), sunflower cake (29.64%), goat manure (28.77%), and cotton seed cake(23.90%). Rests of the amendments groundnut cake and poultry manure were found comparatively less effective with reduction percent wilt recorded was from the range of 20.98 to 8.65 per cent.

Average reduction in the incidence (PEM and Wilt) recorded in the test amendments were ranged from 8.17 to 60.66 per cent over untreated control.

However, significantly highest reduction was recorded with vermicompost (60.66%), followed by karanj cake (53.74%),neem seed cake (46.7 %), compost (41.33%), sunflower cake (29.98%), goat manure (27.20%) and cotton seed cake (22.87%).

Rests of the amendments groundnut cake and poultry manure were found comparatively less effective with reduction in average incidence was range from 16.90 to 8.67 per cent.

Results of the present study obtained on efficacy of organic amendments *viz.*, vermicompost, karanj cake, neem seed cake, compost, sunflower seed cake, goat manure, cotton seed cake, groundnut cake and poultry manure against *R. solanacearum* are in conformity with those reported earlier by several workers (Sharma and Kumar, 2000; Sharma and Kumar, 2004; Islam and Toyota, 2004; Bose *et al.*, 2004;Messiha *et al.*, 2007; Sharma and Kumar, 2009;Ghosh *et al.*, 2009.,Yadessa *et al.*, 2010; Reddy *et al.*, 2012 and Djeugap *et al.*, 2014)

Table.1 Efficacy of organic amendments against *R. solanacearum*

Tr. No	Treatments	Germination* (%)	Incidence* (%)		Av. (%)	Reduction over control (%)		Av. (%)
			PEM	Wilt		PEM	Wilt	
T_1	Compost	61.67 (51.75)	38.33 (38.25)	40.59 (39.58)	39.46 (38.92)	39.52 (38.95)	43.15 (41.06)	41.33 (40.01)
T_2	Poultry manure	40.00 (39.23)	60.00 (50.77)	65.27 (53.89)	62.63 (52.32)	7.69 (16.10)	8.65 (17.10)	8.17 (16.61)
T_3	Vermicompost	76.50 (61.00)	23.33 (28.88)	30.55 (33.55)	26.94 (31.27)	64.10 (53.19)	57.22 (49.15)	60.66 (51.15)
T_4	Goat manure	51.55 (45.89)	48.29 (4.024)	51.51 (45.87)	49.90 (44.94)	25.63 (30.42)	28.77 (32.44)	27.20 (31.44)
T_5	Karanj cake	71.74 (47.89)	28.40 (32.20)	34.92 (36.22)	31.66 (34.24)	56.40 (48.68)	51.09 (45.62)	53.74 (47.14)
T_6	Groundnut cake	43.29 (41.14)	56.67 (48.83)	61.57 (51.69)	59.12 (50.25)	12.82 (20.98)	20.98 (27.26)	16.90 (24.27)
T_7	Sunflower cake	56.72 (48.86)	43.41 (41.21)	50.25 (45.14)	46.83 (43.18)	33.33 (35.26)	29.64 (32.99)	29.98 (32.20)
T_8	Cottonseed cake	48.25 (44.00)	51.67 (45.96)	55.55 (48.19)	53.61 (47.07)	21.84 (27.86)	23.90 (29.27)	22.87 (28.27)
T_9	Neem seed cake	66.75 (54.79)	33.25 (35.21)	36.75 (37.32)	35.00 (36.27)	48.72 (44.27)	45.20 (42.25)	46.7 (43.11)
T_{10}	Control (Untreated)	35.00 (36.27)	65.00 (53.73)	71.42 (57.68)	68.21 (55.68)	--	--	--
	SE ±	**1.75**	**1.75**	**2.76**	**2.25**	**2.15**	**2.40**	**2.27**
	CD (P= 0.01 %)	**5.21**	**5.21**	**8.24**	**6.75**	**6.41**	**7.14**	**6.82**

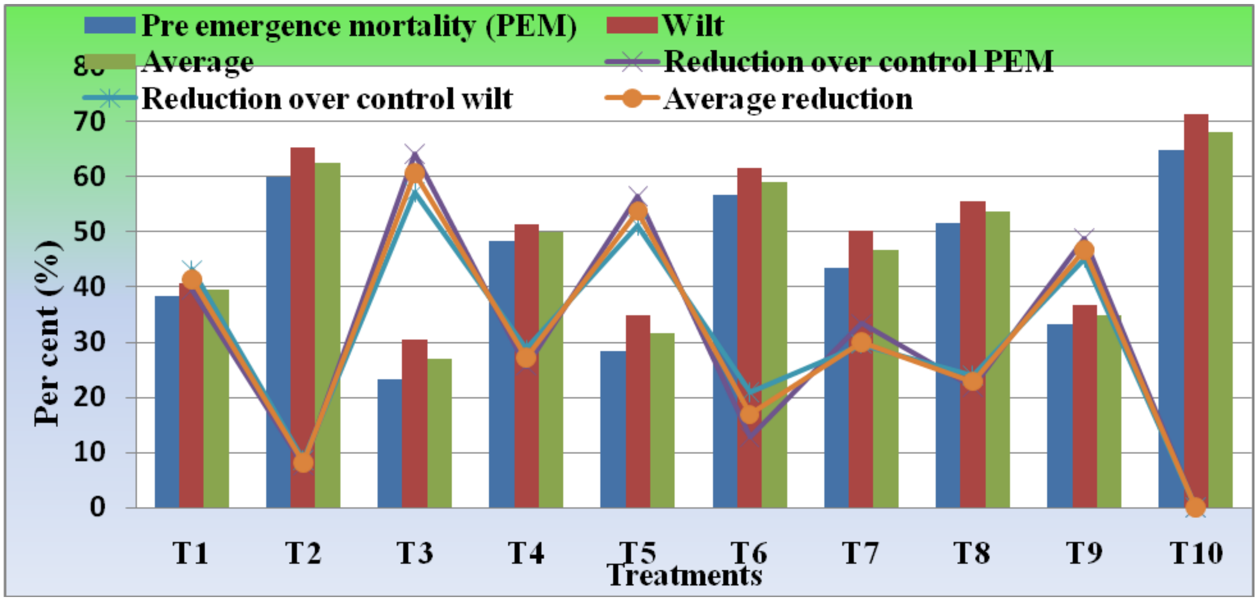
*Means of three replications, Figures in parenthesis are arcsine transformed value, Av = Average, PEM = Pre emergence mortality

Table.2 Reactions of tomato genotypes, germplasm lines, cultivars and varieties against *R. solanacearum*(pot culture)

Tr. No	Treatment /Entries	Disease incidence (%)		Average incidence	Varietal reactions
		30 DAT	45DAT		
T1	Tom -15	33.33	65.33	49.33	MS
T2	Tom -11	67.72	37.17	52.46	MS
T3	Tom -21	24.12	30.00	27.06	MR
T4	Tom -8	63.50	67.20	65.35	S
T5	Tom -27	67.17	33.55	50.33	MS
T6	Tom -7	26.50	69.13	47.81	MS
T7	Tom -4	29.33	31.83	30.58	MR
T8	Tom -18	59.43	62.83	61.13	S
T9	Tom -2	65.00	67.83	66.41	S
T10	Tom -17	22.23	28.33	25.28	MR
T11	Tom -13	22.13	33.33	27.73	MR
T12	S-22	27.50	32.00	29.75	MR
T13	PKM-1	31.37	35.13	33.25	MR
T14	Pusa ruby	79.00	78.00	78.5	S
	SE ±	**0.75**	**0.40**	**0.57**	**--**
	CD	**2.24**	**1.17**	**1.71**	**--**

DAT- Days after transplanting, S- Susceptible, MS – Moderately susceptible and MR- Moderately resistant

Fig.1 In vitro efficacy various organic amendments against *R.solanacearum*



Evaluation of tomato test entries against *R. solanacearum*

Results (Table 2) revealed the under artificial epiphytotics and controlled conditions of the screen house, all 14 tomato entries exhibited different reactions against *R solanacearum*. However, six test entries Tom-21, Tom-13, Tom-17, S-22 and PKM -1 were found moderately resistant with average bacterial wilt percent disease incidence in the range from 25.28 to 50.33per cent; while four test entries Tom-7,Tom-15, Tom-11 and Tom- 27 were found moderately susceptible to bacterial wilt with average percent disease incidence in the range from 47.81 to 52.46 per cent; whereas, four test entries Tom-8, Tom-18 Tom-2 and Pusa Ruby found susceptible with average percent wilt incidence in the range 61.13 to 78.5 percent. Pusa Ruby was found susceptible to the disease bacterial wilt with maximum per cent disease incidence (64.36 %). None of the entry was found highly resistant or immune to the bacterial wilt of tomato disease. These results obtained on varied reactions of the tomato test entires against *R solanacearum* are on the same line as to that of reported earlier by several workers (Khan *et al.*, 1974; Kapoor *et al.*, 1991; Singh and Sood, 2003; Sudheendra *et al.*, 2003; Biswas and Singh 2007; Matsunga *et al.*, 2011;Myint, 2011; Artal *et al.*, 2012; Dutta and Rahman 2012; Dutta *et al.*, 2013 and Pawaskar *et al.*, 2014).

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