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Aakash
Department of Plant Pathology, CCS Haryana Agricultural University, Hisar, Haryana, India

Sandeep Kansal
Department of Plant Pathology Dr. Y.S. Parmar University of Horticultural and Forestry, Himachal Pradesh, India

Ashwani Kumar
Department of Plant Pathology, CCS Haryana Agricultural University, Hisar, Haryana, Haryana, India

Anil Kumar Saini
Department of Plant Pathology, CCS Haryana Agricultural University, Hisar, Haryana, Haryana, India

Garima
Department of Plant Pathology, CCS Haryana Agricultural University, Hisar, Haryana, Haryana, India

Corresponding Author:
Aakash
Department of Plant Pathology, CCS Haryana Agricultural University, Hisar, Haryana, India

Management of tomato bacterial wilt of tomato incited by *Ralstonia solanacearum*

Aakash, Sandeep Kansal, Ashwani Kumar, Anil Kumar Saini and Garima

Abstract
Tomato bacterial wilt incited by *Ralstonia solanacearum* is regarded as a major disease of solanaceous vegetables being its wide distribution in tropical, subtropical and in some warm temperate regions. The study was conducted at Plant Pathology laboratory, Dept. of Plant Pathology, Dr. Y.S. Parmar University, Solan, Himachal Pradesh to find out the efficacy of different chemicals and bioagents against bacterial wilt of tomato. Seven chemicals *viz*: Blitox, Kocide, Nordox, Bordeaux mixture, Streptocycline, Agrimycin and Bleaching powder were evaluated at three different concentration sunder *in-vitro* conditions. The present investigation depicted streptocycline as potent inhibitor of *R. solanacearum* while the *in vivo* studies revealed the significant efficacy of streptocylcine @ 200 ppm applied as seed and seedling root dip treatment in combination with three periodic drenching with Bordeaux mixture @ 0.8%. Field evaluation studies revealed treatment consisting of application of bleaching powder @ 15 kg/ha before transplanting, amending the soil pH to neutral with lime, seedling root dip treatment with streptocycline @ 200 ppm for 5 minutes in combination with four periodic drenching with Bordeaux mixture @ 0.8% at 10 days interval starting with appearance of disease as efficacious in limiting the bacterial wilt (65.47%) and enhancing the fruit yield (380.7 q/ha).

Keywords: Bacterial wilt, chemical, bioagents, tomato, management

Introduction
Tomato (*Lycopersicon esculentum* L.) is an important solanaceous vegetable crop cultivated throughout the word. Owing to its perennial cultivation on large scale and perishable nature, it remains susceptible to attack by several plant pathogens leading to poor yield (Tajul, *et al*. 2011) [10]. Among the various pathogens, *Ralstonia solanacearum* (Smith) Yabuuchi *et al*., inciting bacterial wilt is of prime importance, posing significant economic impact on sound cultivation of tomato crop throughout the world (Scherf *et al*., 2010) [8]. The pathogen manifest by producing symptoms of leaf epinasty and browning of vascular bundles (Yahiaoui, 2016) [13]. The etiological studies indicated that pathogen invade through plant roots followed by colonizing the intercellular spaces of roots, eventually progressing towards xylem vessels, leading to wilting symptoms (Hikichi *et al*., 2017) [1]. Planting tomato plants harbouring genetic resistance to bacterial wilt have been suggested for desirable and durable tomato productions (Jung *et al*., 2014; Kim *et al*., 2016; Lee *et al*., 2015) [2, 4]. However, the cultivar resistance of tomato plants to bacterial wilt can be overcome by newly emerging pathogenic bacterial strains and changing climates such as elevated temperatures and ambient CO_2 concentration (Lee *et al*., 2011) [4]. Although, for the control of bacterial wilt disease in different crops, several attempts were made in the area of biological control with varied levels of success but there is still a great opportunity to provide a stable solution to this problem (Bailey and Lazarovits 2003) [14]. Therefore, there is need to develop an integrated module that could effectively manage the disease. The present study is planned to find out effective management strategy for bacterial wilt involving the use of reduced risk chemicals and biological control agents.

Materials and Methods
Collection and isolation of pathogen
The diseased plant samples collected during surveys were subjected for microscopic examination to confirm the association of the bacterium. The samples showing the presence of ooze and vascular discoloration were selected for isolation. For this purpose the stem and roots of diseased plants were washed thoroughly in running tap water and small bits of 2 mm were taken from infected portion after proper surface sterilization with sodium hypochlorite (5%)

solution. Then bits were added in sterilized distilled water and placed on shaker for 2-3 days until suspension became translucent. Then loop full of bacterial cell suspension was streaked on Petri plates containing the nutrient agar medium and incubated at 28±1 °C. After 36-72 h, the Petri plates were examined and culture was further purified and multiplied on triphenyl tetrazolium chloride basic medium, a specific medium for the growth of *Ralstonia solanacearum*.

In vitro* evaluation of different chemicals against *Ralstonia solanacearum

Seven chemicals Blitox 50 WP (1000, 2000, 3000 ppm), Kocide 3000 (1000, 2000, 3000 ppm), Nordox (1000, 2000, 3000 ppm), Bordeaux mixture (1000, 2000, 3000 ppm), Streptocycline (150, 200, 250 ppm), Agrimycin (150, 200, 250 ppm) and Bleaching powder (1000, 2000, 3000 ppm) were evaluated for their efficacy against *Ralstonia solanacearum* under *in vitro* conditions by disk diffusion method (Murray *et al;* 1995, modified by Olurinola *et al*; 1996) [6, 7]. One ml of standard bacterial suspension ($3x10^8$ c.f.u/ml) was mixed with nutrient agar medium (20 ml) in sterilized Petri plates and allowed to solidify. The filter paper disc of 5 mm in diameter was soaked to the chemical suspension of required concentration and placed on the solidified and seeded nutrient agar Petri plates. Each treatment was replicated five times. These Petri plates were incubated at 28 ± 1 °C for 48 hours and observed for the production of inhibition zone around the filter paper discs.

***In vivo* evaluation of chemicals and bio agent**

To study the efficacy of chemicals & bioagents in managing bacterial wilt, the four week old seedlings were transplanted in plastic pots (7.5 cm dia.) filled in with sterilized soil. After 7 days of transplanting the plants were drench inoculated (50 ml/kg of soil) with the nutrient broth culture (48 hrs old) of the test bacterium ($3x10^8$ c.f.u/ml) and incubated at 30±2 °C in relative humidity cum temperature control cabinet. A total number of seven treatment consisting of seed & seedling dip treatment and drenching with chemical and bio agent were evaluated in the present study. The details of treatment applied as T_1 = Control; T_2 = Seed treatment and seedling root dipping with streptocycline @ 200 ppm; T_3 = Seed treatment (10 g/kg) and seedling dip with *Pseudomonas fluorescence* @ 1%; T_4 = Drenching of Bordeaux mixture @ 0.8% thrice at 7 days interval after 10 days of transplanting; T_5 = Drenching of *Pseudomonas fluorescence* @ 1% thrice at 7 days interval after 10 days of transplanting; $T_6 = T_2 + T_4$; and $T_7 = T_3 + T_5$. Ten plants were taken per replication and the treatments were replicated three times. The data on bacterial wilt disease incidence were recorded as per scale given by Winstead and Kelmen (1952) [11].

$$\text{Disease incidence} = \frac{\text{Total number of wilted plants}}{\text{Total number of observed plants}} \text{ x } 100$$

Evaluation of chemicals (fungicide, antibiotic, disinfectant) & bioagent

In order to study the effect of treatments given through different types of chemicals and a bio agent against bacterial wilt of tomato on variety Himsona, a field trial was laid out in sick soil of farmer field at Pandah in Randomized Block Design with the plot size of 2.7 x 1.8 m^2 with three replications. The treatments consisted of chemicals and a bio agent which were applied during pre-sowing /sowing/post transplanting or at all the stages. The details of treatment applied: T_1 = Soil application of bleaching powder @ 15kg/ha before transplanting; T_2 = Soil amendment with lime depending upon pH of the soil to make soil neutral; T_3 = Seedling root dipping with streptocycline @ 200 ppm; T_4 = Drenching of Borodeaux mixture @ 0.8% four times at 10 days interval starting after initiation of disease; T_5 = Seed treatment (10 g/kg) and seedling root dip @ 1% with *Pseudomonas fluorescence*; T_6 = Drenching of *Pseudomonas fluorescence* @ 1% thrice at 10 days interval started after initiation of disease; and $T_7 = T_1 + T_2 + T_3 + T_4$; $T_8 = T_2 + T_5 + T_6$; and T_9 = Control.

Observations on bacterial wilt disease incidence and fruit yield in each treatment per plot were taken to work out the relative efficacy of treatment.

Results and Discussions

Morphology and pathogenicity of *R. solanacearum*

The test bacterium was also purified on triphenyl tetrazolium chloride (TZC) medium, which is a specific medium for the growth *Ralstonia solanacearum.* The test bacterium produced fluidous colonies which were generally smooth, round with white margin and reddish-pink centre after 36h of incubation at 28±2 °C (Fig. 1).

Fig 1: Culture of *Ralstonia solanacearum* retrieved from wilted plant

In vitro* evaluation of chemicals against *Ralstonia solanacearum

Seven chemicals were tested against *Ralstonia solanacearum* under *in vitro* conditions through paper disc method. The data regarding the zone of inhibition by different chemicals at different concentrations levels is given in Table 1. The study (Table 1) revealed variable inhibition responses of different chemicals against *Ralstonia solanacearum* causing bacterial wilt of tomato.

Table 1: *In vitro* efficacy of different chemicals against *Ralstonia solanacearum*

Chemicals	Growth inhibition (mm^2) at concentrations			Mean
	C_1	C_2	C_3	
Blitox-50 WP*	4.10	7.28	10.30	7.23
Kocide 3000*	5.70	8.80	13.80	9.43
Nordox*	4.90	7.80	13.10	8.60
Bordeaux mixture*	6.30	10.9	14.5	10.57
Streptocyline**	8.24	14. 26	20.50	14.37
Agrimycin**	8.15	13.79	16.19	12.71
Bleaching powder*	6.1	10.2	14.10	10.13
Mean	6.21	9.80	14.64	

*Concentration C_1, C_2, C_3 used were 1000, 2000 and 3000 ppm, respectively
**Concentration C_1, C_2, C_3 used were 150, 200 and 300 ppm, respectively
$CD_{0.05}$ Chemical = 0.27, Concentration = 0.18, Chemical x Concentration = 0.47

Further perusal of data (Table 1) revealed that irrespective of different dosages level tested, streptocycline proved superior resulting in maximum zone of inhibition (14.37 mm^2) followed by agrimycin (12.71 mm^2). Bordeaux mixture, bleaching powder & Kocide 3000 resulted in moderate inhibition while Blitox-50 was least inhibitory. Irrespective of different chemicals tested for their *in vitro* inhibitory effect against *Ralstonia solanacearum*, the increasing inhibitory response was observed with increasing dosages levels of the chemicals.

***In vivo* evaluation of chemicals and bio agents against bacterial wilt of tomato under pot culture experiment**

It is evident from the data (Table 2) that different treatment resulted in reduction of bacterial wilt incidence to varied extent. The treatment (T_6) consisting of seed and seedling root dip treatment with streptocycline @ 200 ppm in combination with three drenching with Bordeaux mixture @ 0.8% at 7 day interval started after 10 days of transplanting proved most efficacious in limiting the bacterial wilt. The treatment (T_4) i.e. drenching with Bordeaux mixture & (T_7) consisting of seed and seedling root dip treatment with *Pseudomonas fluorescence* @ 1% & its periodic drenching were next efficacious. Independent usage either of streptocycline (T_2) or *P. fluorescence* (T_3) for seed and seedling root dip treatment were found efficacious to lesser extent.

Table 2: *In vivo* evaluation of chemicals and bio agents against bacterial wilt of tomato under pot culture

Treatment	Percent Disease index after days of transplantation			Mean
	14	21	28	
T_1 – Control	35.8 (36.75)	76.40(60.94)	100.00(90.00)	70.73(62.56)
T_2 – ST & SRD streptocycline @ 200 ppm	20.6(26.99)	54.6(47.64)	78.2(62.17)	51.13(45.60)
T_3 - ST & SRD *P. fluorescens* @ 1%	26.7(31.11)	59.2(50.30)	82.6(65.35)	56.17(48.92)
T_4 – Drenching Bord. Mix @ 0.8%	14.7(22.55)	36.4(37.11)	52.4(46.38)	34.50(35.35)
T_5- Drenching *P. fluorescens* @ 1%	18.8(25.70)	42.9(40.92)	64.6(53.49)	42.10(40.04)
T_6 – T_2 + T_4	9.4(17.85)	28.6(32.33)	44.8(42.02)	27.60(30.73)
T_7- T_3+T_5	12.8(20.96)	38.5(38.35)	56.2(48.56)	35.83(35.96)
Mean	19.83(25.99)	48.09(43.94)	68.40(58.28)	

Values given in brackets are the arcsine transformed values
$CD_{0.05}$ Treatment = 2.70, Interval = 1.77, Treatment x Interval = 4.69

Evaluation of chemicals and bio agents against bacterial wilt of tomato under field conditions

The results of the study (Table 3) indicated that different chemical and non-chemical treatments were effective against bacterial wilt of tomato to a variable extent. Among stall the treatments, the treatment combination (T_7) consisting of soil amendment with lime, soil application of bleaching powder @ 15 kg/ha before transplanting, seedling root dipping with strep to cycling @ 200 ppm in combination with four periodic drenching with Bordeaux mixture @ 0.8% at 10 days interval started after initiation of disease proved most efficacious in limiting (65.47%) the bacterial wilt on tomato and enhancing the fruit yield (380.7 q/ha). The treatment (T_4) i.e. periodic drenching with Bordeaux mixture @ 0.8% and treatment (T_8) i.e. soil amendment with lime in combination with seed and seedling root dip treatment with *Pseudomonas fluorescens* & its four periodic drenching @ 1% being similar in their efficacies were rated next efficacious. Pre sowing application of bleaching powder @ 15 kg/ha also provided significant control (45.64%) of the disease. Independent usage of streptocycline and *Pseudomonas fluorescens* for seed and seedling root dip were effective to lesser extent while soil amendment with lime was least effective.

Table 3. Efficacy of different chemical & non chemical methods against bacterial wilt of tomato under field conditions

Treatment	Bacterial wilt incidence (%)			Disease reduction (%)	Mean yield (q/ha)	Increase in yield (%)
	30 DAP	60 DAP	90 DAP			
T_1-SA Bleaching Powd. @ 15 kg/ha	1.0 (5.74)	12.2 (20.44)	45.5 (42.42)	45.64	256.7	85.21
T_2- Soil application Lime	2.1 (8.33)	17.7 (24.88)	64.4 (53.37)	23.06	158.6	14.43
T_3- SRD streptocyc@200 ppm	1.0 (5.74)	15.5 (23.19)	58.0(49.60)	30.70	198.2	43.00
T_4- Bord. Mix. Drenching @ 0.8%	0.0(0.71)	9.25 (17.71)	38.2(38.18)	54.36	304.4	119.62
T_5- ST &SRD P f @1%	1.0 (5.74)	17.7 (24.88)	63.7(52.95)	23.89	160.2	15.58
T_6- P f Drenching @1%	1.0 (5.74)	15.5 (23.19)	45.6(42.48)	45.52	254.9	83.91
T_7- $T_1 + T_2 + T_3 + T_4$	0.0 (0.71)	3.3 (10.47)	28.9 (32.52)	65.47	380.6	174.60
T_8-$T_2 + T_5 + T_6$	0.0(0.71)	11.0 (19.37)	40.6(39.58)	51.49	296.8	114.14
T_9	7.6(16.0)	33.2 (35.18)	83.7(66.19)	-	138.6	-
CD($_{0.05}$)	0.63	2.14	2.80		37.25	

Conclusion

Bacterial wilt of tomato caused by *Ralstonia solnacearum* (Smith) Yabuuchi *et al*. appearing in moderate to severe form in different tomato growing localities was observed as a threat to the tomato cultivation in these districts. The bacterium isolated from infected plants was identified as *Ralstonia solnacearum* (Smith) Yabuuchi *et al*. on the basis of morphological, cultural and biochemical tests. The test pathogen manifested initial symptoms as loss of turgidity within 4-5 days of inoculation of 3 week old seedlings which further progressed to drooping/wilting of plants accompanied with vascular discoloration. The *in vitro* evaluation studies indicated streptocycline as potent inhibitor of *R. solnacearum* while the *in vivo* studies revealed the significant efficacy of streptocylcine @ 200 ppm applied as seed & seedling root dip treatment in combination with three periodic drenching with Bordeaux mixture @ 0.8%. Field evaluation studies revealed treatment consisting of application of bleaching powder @ 15 kg/ha before transplanting, amending the soil pH to neutral with lime, seedling root dip treatment with streptocycline @ 200 ppm for 5 minutes in combination with four periodic drenching with Bordeaux mixture @ 0.8% at 10 days interval started with appearance of disease as efficacious in limiting the bacterial wilt (65.58%) and enhancing the fruit yield (380.7 q/ha).

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